

Impaled on Morton's fork

British archaeologists, bent on organizing a successful international conference next year, should have found a more honourable way out of their problem over South African apartheid.

By the time this issue of *Nature* reaches its readers, the British National Committee of the International Union of Prehistoric and Protohistoric Sciences (IUPPS) will have made the most difficult decision ever to have come its way. For the best part of a year, the committee has been planning the 11th World Archaeological Congress, arranged for Southampton next September (see *Nature* 31 October, p. 754). Just two months ago the organizers found themselves in what they judged to be an impossible position. Local interests at Southampton had complained that they would boycott or even disrupt the congress if scientists from South Africa were allowed to attend. The committee seems to have spent the summer agonizing about the problem, and the obvious conflict there would be between a decision to give in to opponents of the conference and the ringing declaration of the parent body, IUPPS, there should be no discrimination in its proceedings on the grounds of "nationality, philosophical conviction or religious faith". The issue was further complicated by the circumstance that the chairman of the British organizing committee, Professor John Evans, is also president of IUPPS. But in the end, the organizers decided that they had better bow to pressure to disinvite South Africans. A note to that effect was included in the final version of the conference programme.

This week's meeting (on 20 November) has the unavoidable purpose of mulling over the damage that has been done to international scholarship, to the reputation of British academic institutions and, more practically, to the chances that the planned congress will be a success, financially as well as intellectually. There are three choices: to stand by the decisions already made, to go back on them (letting the opposition go hang) or to cancel the congress (perhaps in the hope that it might be possible to hold it elsewhere at some other time). It probably matters very little what the committee decides. Most of the damage has already been done.

The rights and wrongs of the underlying issue are widely understood. The South African system of apartheid, one of the few systems of racial discrimination enshrined in a nation's constitution, is wicked, and should be done away with. Most people agree with that. In the past few years, external pressure has helped to moderate the South African government's policies, and there is now a chance that further pressure will make the system of apartheid untenable. That proposition (or package of propositions) is less unanimously accepted. Plain speaking certainly helps. Economic sanctions have dubious direct effect, but may have symbolic value. The steps taken by the governments of the British Commonwealth under the Glencagles agreement to make it difficult for sportsmen who play games in South Africa then to play the same games for their own representative teams have been an endless source of trouble but are probably nevertheless worthwhile, given South Africa's fondness for most kinds of games. But the pressures to which the organizers of the Southampton congress have been subjected appear to derive from the belief that if South African scientists are denied the chance to play a part in international conferences, their government will similarly cave in. To say the least of it, that proposition is untested.

Whatever the chances, there seems no doubt that IUPPS and its British organizing committee should have resisted the pressures

to which it was itself exposed during the summer. IUPPS is not, after all, the government of South Africa. Its constitution does include the stirring declaration that its proceedings are open to all. The British committee's two public statements of its reasons for disinviting South Africans, on 19 September and 22 October, refer to the decision as "hard" and as "taken under duress"; the second "keenly regrets the breach of the principle of free academic interchange". The first refers to the "pressure from several official and worthy organizations" in Britain "which maintain a policy of a total academic boycott of South Africa". The second more explicitly explains that if some of the proposed sponsors of next year's congress, the Southampton City Council in particular, were to withdraw support, the organizing committee might lose as much as £100,000, a quarter of the congress budget. Plainly one reason why the decision was so hard to make was that the committee members were forced to swallow their pride for lack of financial independence.

Nobody should take advantage of the committee's plight to throw brickbats at it. There is no reason to suppose that its members share the views of the pressure groups complaining that South Africans might attend the congress, and which have threatened trouble if they do. But it is fair to ask why the committee, conscious as it appears to have been of the principles of academic freedom, should have caved in so quickly and privately. At the least, it might have made a public issue of its dilemma, pleading the aid of other academic organizations or of academic opinion in general. The committee may have thought it had a duty, in the summer, to act quickly so as to make its conference a success, but by what right did it assume the responsibility of electing itself to be the first academic body to promulgate discriminatory regulations against fellow academics?

For the academic community as a whole, the objective now should be to forfend against future trouble of the kind that has afflicted the British offshoot of IUPPS. The Southampton City Council, the largest single sponsor of the planned conference, seem to have influentially twisted the organizers' arms; the lesson is that academics should not accept support if there are, or may be, strings attached. Student organizations seems to have been influential at Southampton in threatening to deny accommodation to visitors if South African scholars were among them, but it is far from clear how students to whom universities rent accommodation during term-time can decide what happens to it during the vacations, when they pay no rent. But particular responsibility for the IUPPS dilemma, and perhaps even for the collapse of a worthwhile conference, attaches to the Association of University Teachers, which has pursued a policy of "academic boycott" in relation to South Africa; is that what British academics wish their representative body to be saying?

To resist these pressures would of course be inconvenient. Congresses would be less grand if city councils were less generous, and would be less well attended if there were not cheapish student accommodation in which people could stay. There is also a danger that scientists from other countries would stay away if South Africans were free to attend, impeding the process of scientific communication. But scientific organizations are not powerless in the face of such difficulties. Plain speaking, and persuasion in the interests of tolerance, are freely at their disposal. They are tools too little used. □



US science budget

Congress sets upper limits for federal agencies

Washington

THE US Congress has not done as badly by research this year as many had feared it would. In spite of the strong political pressures to be seen doing something to reduce the federal budget deficit, attempts to freeze some appropriations at 1985 levels were defeated. But Congress conspicuously failed to meet the administration's budget requests for several agencies in the new financial year. The National Science Foundation (NSF) and the National Aeronautics and Space Administration (NASA) are among the casualties.

NASA's budget for research and development in the year which began on 1 October is fixed at \$2,757 million, the figure that had been urged by the House of Representatives; the Senate had wanted an extra \$20 million. The sum now agreed represents an increase of \$335 million over last year's figure, but is significantly less than the \$2,881 million the administration had requested.

Specifically, Congress has denied the administration's request for \$226 million for development of the space station, putting a cap on the level of spending next year at \$205 million. Of this, \$5 million is set aside for the robotics and automation thought necessary for the initial operating capability of the space station. Congress seems concerned that budgetary restrictions in future might hold up space science if the administration persists in its plans for a permanently manned structure from the start.

A cap of \$5 million (with a further \$5 million available upon request) has also been put on development of the solar optical telescope, a 1.25-metre instrument that would form the basis of a solar observatory on the space station. This will mean a significant delay for the project, for which \$30 million had been sought in 1986. Other capped projects include: upper stages (\$122 million); the space telescope (\$127 million); the gamma-ray observatory (\$87 million) and the Galileo spacecraft (\$40 million). The funds allotted to space flight, control and data communications are \$3,398 million, about \$100 million less than requested.

The total appropriated for research and related activities by NSF is \$1,352 million, an increase of about \$40 million over last year. The administration's request had been for \$1,398 million. The conference report of the appropriation bill dealing with NSF (in which differences between the House and Senate are patched up) specifically notes that sufficient resources must be made available to support advanced scientific computing and the ocean

drilling programme, as well as efforts to increase women's and minority participation in science and engineering.

NSF is also given \$115 million for the US Antarctic programme and \$55.5 million for science education, which represents a compromise between the figures urged by the Senate and the House. The foundation also has (to Congress's displeasure) \$31.5 million in its science education budget unspent from last year, so that the total for this purpose will be \$87 million, a dramatic increase on what was spent last year. The Very Long Baseline Array is, however, capped at \$9 million;

astronomers had hoped for \$11.5 million for the project. The increase in NSF's total budget, at close to 2 per cent is in striking contrast to the 15 per cent increase it got the year before.

Congress has also for the first time in several years, passed an authorization bill for NSF. The authorization may have already been influential in persuading Erich Bloch, NSF's director, to restore a \$1 million programme on ethics and values in science and technology which he had previously planned to axe. Otherwise, the bill will have little immediate effect, although it is likely to focus attention on particular areas such as fundamental engineering research and research facilities in universities, which NSF is now directed to study systematically. The bill will also give the National Science Board the authority to delegate grant approval authority to the director.

Tim Beardsley

British research funds

Government helps to plug dyke

BURIED in the British government's disappointing announcement last week of its spending plans for higher education and research in 1986-87 is some good news for the research councils, which will have an extra £15 million a year to spend. This is seen to result partly from a sombre report on the loss of British scientists overseas prepared by the chairman of the Advisory Board for the Research Councils (ABRC), Sir David Phillips, and published last week.

The Chancellor of the Exchequer's autumn budget statement last week makes it plain that the recurrent budget of the universities is to be held at the levels proposed in January this year, which have been estimated to entail a cut of 1.6 per cent in real terms. Specifically, the university budget will be 2.5 per cent greater in cash terms next year than this, a rate of increase less than the inflation rate.

The research councils are dealt with more generously. The budget statement says that there will be an extra £15 million a year for each of the next three years, in addition to last year's emergency grant of £27 million (spread over three years). A further £10 million will be distributed to selected university research centres for the purchase of equipment.

The immediate explanation of the government's generosity seems to be that Sir Keith Joseph, Secretary of State for Education and Science, has been persuaded of the reality of the rate at which technically qualified people are being lost to Britain. ABRC has been warning the government regularly about the numbers of scientists moving overseas, but the government has repeatedly dismissed the claim, made most forcefully in ABRC's *Science and public expenditure* statement earlier this year, on the grounds that the

evidence is "anecdotal".

ABRC has therefore carried out a survey among 40 leading research groups in Britain (38 in universities and two at Medical Research Council (MRC) units). It now says that its data reinforce its "firm impression" that there is a serious loss of talent, not only by emigration elsewhere but also to industry and to careers in Britain not based on science.

According to ABRC, the reasons given for the loss of talent are "remarkably consistent": more opportunities elsewhere, better pay and prospects, better facilities, frustration about difficulties in winning research grants in Britain, aggressive recruiting by overseas employers and the greater receptiveness of US industry to new ideas.

Although none of these is new, scientists agree that the gap between the United States and Britain has widened in the past few years. Dr Sydney Brenner, director of the MRC Molecular Biology Laboratory in Cambridge, told ABRC that British university research (with a few exceptions) has not been able to keep pace with international standards and has often declined to a point from which recovery is impossible. Engineering was singled out by ABRC as a discipline in urgent need of support; the two Cambridge engineering departments included in the survey reported that 75 per cent of their recent research students have been lost to overseas appointments.

ABRC has not yet decided how to divide Sir Keith's extra money among the four research councils. But as long as the government continues its last-ditch approach to science funding, the brightest scientists are unlikely to prefer an uncertain home career to the tangible rewards elsewhere.

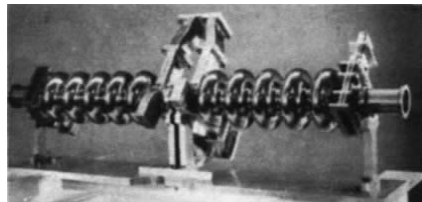
Maxine Clarke

US accelerators

Virginia sets its sights higher

Newport News, Virginia

LESS than a year after being appointed director of the Continuous Electron Beam Accelerator Facility (CEBAF), Hermann Grunder is proposing a radical change of design for the accelerator to be built here on a green-field site starting in 1989 — if Congress approves. Grunder wants to abandon the previously-canvassed combination of room temperature linear accelerator and pulse-stretching storage ring and to use instead superconducting



Cornell-CEBAF superconducting niobium electron accelerator element. Each element includes five radio frequency cavities.

niobium radio-frequency cavities to build a continuous-wave electron accelerator, which is claimed to give superior performance for the same cost.

Part of the interest of the project is political; two years ago, the Southern Universities Research Association stole the project from under the nose of the Argonne National Laboratory. Technically, its importance is that it will be the only source of virtually continuous electron beams in the GeV range.

The proposal is being presented this week to the Department of Energy, which will decide by 1 December which design to support. The new design would consist of two antiparallel 0.5 GeV linacs connected end to end by arcs; electrons would circulate around the ring 4 times for a maximum design energy of 4 GeV. That the accelerator should produce a beam that is almost continuous in time is central to its purpose, as such a beam allows "coincidence" measurements to be made; the new design produces a continuous beam directly rather than relying on a pulse-stretching ring.

The advantages of the new design compared with the old are that the energy spread of the electrons will be reduced by a factor of 10; emittance (a measure of beam size) will be reduced by a factor of 100; and power consumption will be reduced by a factor of three, to 6 MW, because of the much lower energy losses in superconducting magnets. Furthermore, electrons will at any time be available at four different energies, corresponding to the number of revolutions completed.

Grunder was recruited to CEBAF from Lawrence Berkeley Laboratory at the beginning of this year, when enthusiasm for the project was waning. The idea of a high-duty cycle accelerator working in the 0.5–4 GeV energy range has been talked

about in the nuclear physics community since 1976, but it was not until 1983 that the CEBAF proposal submitted by the Southern Universities Research Association was recommended to the government for development funding, after a bitterly contested fight with Argonne National Laboratory. Grunder was tempted to the post of director with assurances that substantial research funds would be available in 1987 and 1988 so that he could conduct a complete technology review.

Grunder describes the superconducting continuous wave design as a "responsible risk" proposal. The concept was first demonstrated in the 1960s; advances in technology since then have made it a desirable alternative that has been investigated at several accelerator research centres and developed most recently at Cornell University. Commercial manufacturers are already able to supply the high-precision high purity niobium cavities used.

The design target adopted is 5 MeV per

metre, but in tests some cavities have already performed well above this level. The cryogenic system, which would operate with low-pressure liquid helium at 2K, is large but well within commercial manufacturers' present-day capabilities, according to Grunder. And the need for very high power klystrons, which were the target of a major development effort for the old design, is avoided altogether. Grunder estimates that the new design can be built for the same or slightly less than the \$236 million price-tag for the previous proposal.

The design energy of 4 GeV will enable quark cluster in the nucleus to be examined, but to look for semi-free quarks many physicists believe higher energies will be necessary. The new design could easily be upgraded later to increase energies two or threefold. Grunder is confident that he will be able to sell his new design to the Department of Energy and to Congress: he would, he says, rather be introducing a new design option even at this late stage than fielding questions about why the superconducting option was not considered.

Tim Beardsley

Embryo research

Chances of legislation fade

MR Enoch Powell MP's Unborn Children (Protection) Bill, already killed twice in the British House of Commons this year, has spluttered into life again, although its chances of prolonged survival are slim. Powell's bill will ban *in vitro* manipulations of human embryos, excepting only cases where a particular pregnancy is to be brought to term. If the bill becomes law, Britain's *in vitro* fertilization programme would halt.

Since the death of the bill, popular with many MPs, by filibuster, Mr Barney Hayhoe, a supporter of the bill, was appointed Minister for Health in the summer cabinet reshuffle, in succession to Mr Kenneth Clarke, an opponent. Hayhoe's personal views, however, are unlikely to lead him to introduce legislation that the Prime Minister, Mrs Margaret Thatcher, is known not to support.

Mrs Thatcher is understood to favour legislation to implement the recommendations of the Warnock Committee, published last year after two years of deliberations. But the government's legislative programme for the coming years, embodied in the Queen's Speech two weeks ago, says nothing about the subject, suggesting that a government bill will not make its appearance for at least a year.

So opponents of embryo research are again left to the device of the Private Member's bill, the draw for which took place last week. Powell himself drew 13th place in the ballot, so will be unable to introduce his bill himself. But several of the 20 names in the ballot are supporters, and one of these, Mr Tom Clarke, drew

first place. But Clarke prefers a less contentious subject that stands more chance of success. Although Powell's bill had a majority of support in the Commons when originally debated, MPs now seem to have moderated their views.

Part of the explanation seems to be the emergence of a group called *Progress*, including physicians and medical organizations who are seeking to educate MPs and encourage a more "rational and humane judgement".

Meanwhile, scientists using *in vitro* fertilization techniques have been registering with the voluntary licensing authority set up by the Medical Research Council and the Royal College of Obstetricians and Gynaecologists earlier this year. Eleven centres have been approved by the authority so far; eight more are likely to apply for a licence soon. And evidence that the authority is not just a public-relations exercise is provided by three centres whose initial applications were turned down, two of which have now modified their work to conform with the guidelines and have been given licences, and the third of which is expected to reapply soon. All the approved projects involve embryos less than nine days old and concern chromosome abnormalities; non-destructive identification of optimum growth media and normal stages of growth; and cryopreservation of pre-embryos. The authority will issue its first annual report in March, when it will give details of the research at the approved centres and identify those that have not applied for a licence.

Maxine Clarke

Japanese honours

Prizes, prizes, prizes . . .

Tokyo

THE first recipient of Japan's new International Prize for Biology is an Englishman: Professor Edred Corner, formerly holder of the chair in tropical botany at the University of Cambridge and now, at almost eighty years of age, in active retirement.

The prize was established this year to celebrate the sixtieth anniversary of the accession of the Emperor, himself a noted amateur marine biologist with his own research laboratories. At ten million yen (US \$50,000) in cash plus a medal and a gift from the Emperor, the prize is meant to rank among the international heavyweights. Indeed, to show its importance the Crown Prince, the Prime Minister and the Minister of Education, Culture and Science all turned out to make speeches at the award ceremony. The Emperor too received Professor Corner (who has met him informally in past years) in formal audience at the Imperial Palace.

NIH veto doomed

Washington

CONGRESS seems likely shortly to overturn a presidential veto of the controversial reauthorization bill for the National Institutes of Health (NIH). The bill, which was vetoed by President Reagan on 8 November, would establish a new National Institute of Arthritis, Musculoskeletal and Skin Disease and a new centre for nursing research. It also lays down guidelines on, among other things, how NIH should use peer review for grant applications and on fetal and animal research.

The House of Representatives has already voted by a large majority to overturn the veto; the Senate seems to be in like mind, but a vote will probably be delayed until President Reagan returns from his summit meeting in Geneva.

The President also vetoed an NIH authorization bill last year, but this year's version has commanded stronger support in Congress. Many of the line-by-line budget recommendations in last year's bill have been dropped. And President Reagan has no objection to the creation of the arthritis institute, having directed NIH to go ahead and create the new institute anyway. It will be formed by partitioning the existing National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases.

But the proposed centre for nursing research and a requirement that NIH spend not more than 5.5 per cent of its income on administrative expenses are regarded by the White House as unacceptable. Nevertheless, NIH officials say, many of the administrative recommendations in the bill are likely to be incorporated into NIH practice even if the presidential veto stands.

Tim Beardsley

It is hoped that the prize will become the "Nobel Prize" of biology. This year, the field chosen for the prize was taxonomy; what it will be next year has yet to be decided. But whatever is chosen, it will be critical to the long-term future of the prize that links are made to a vigorous international network of people willing to recommend candidates.

Professor Corner is the author of several standard works of tropical botany much used by Japanese and other researchers visiting South-East Asia and was responsible for a new classification of the genus *Ficus*; he is also a key figure in the establishment of the modern taxonomical systems for fungi.

Although the International Prize for Biology must rate as Japan's most distinguished award, it is not the only prize being given away in Tokyo this week. Professor Carl Sagan is also receiving ¥10 million as part of the Honda Prize set up by the Honda Motor Company. The prize is for "ecotechnology" and Sagan receives it for his contribution toward "ecotechnological exploration of space, as well as toward establishment of a theory of technological development for the new era of civilization based on a more cosmic viewpoint".

There has been a miniature boom this year in establishing new prizes. In the spring, it was the new "Japan prize" which at ¥50 million rivals the Nobel Prize financially and is intended to reward applications of basic science (see *Nature* 315, 616; 1985). A few weeks ago it was the turn of the Kikawada Foundation. This again went to an Englishman, Professor Christopher Freeman, deputy director of the Science Policy Research Unit at the University of Sussex.

But while Japanese companies are generously giving out prizes to foreigners one after another, they are having less luck in finding an agreeable reason for the failure of Japanese scientists to collect top international prizes. Eight officials of the Nobel Foundation, including board chairman Sune Bergstroem, visited Kyoto last week where they received the special prize of the Kyoto Prize. Speculation that the visit would help more prizes to come to Japan, rather than the United States and Europe, were quickly squashed, however. Committee members pointed out that science is international, and that language and distance from Sweden are no barriers to receipt of a prize. An explanation of why a Japanese scientist has never received the Nobel Prize for Physiology or Medicine is rather to be sought in lack of basic research effort. And that might suggest that the new foundations would have served their country better by making up for the lack of funds available for original research in Japan rather than seeking glamour from abroad.

Alun Anderson

French defence

Smaller warheads planned

FRANCE is to develop new miniaturized nuclear warheads to "punch holes" in other people's defence systems, according to the French Minister of Defence, Paul Quilès. In a rare public account of French nuclear policy, Quilès said last week that he had set the Commissariat à l'Energie Atomique several ambitious targets to be achieved by 1994, when the first of a new series of nuclear submarines will come into service.

The miniature warheads are among these objectives, and seem to be a kind of French response to US plans to develop defences against ballistic missiles, the Strategic Defence Initiative (SDI). The more attention the superpowers pay to strategic defence, Quilès said, the more necessary becomes "the penetration capacity of our missiles . . . to our policy of nuclear dissuasion".

Quilès nevertheless repeated last week his government's opposition to SDI. The period when the Soviet Union and the United States were installing the new technology would be a "particularly critical time" for world security, while SDI would be ineffective against the short and medium range missiles aimed at Europe. But it is plain from what he said that France is most of all concerned that the new interest in strategic defence will permit an improvement of the conventional Soviet anti-ballistic missile system already deployed around Moscow. The use of "stealth" technology, both miniaturization and the use of non-reflecting radar coatings, is a natural countermeasure.

In passing, Quilès has also explained French sensitivity about the present programme of nuclear testing in the Pacific, indirectly responsible for the sinking of the *Greenpeace* ship in New Zealand and, ironically, for the resignation of Quilès's predecessor, Charles Hernu. France is obviously committed to a new programme of warhead technology, for which testing is considered essential. In France, there appears to be no opposition to the programme.

From what Quilès said last week, it also seems that the French navy rather than the air force will be the spearhead of the new strategy. Noting that the mere presence of British nuclear submarines in the South Atlantic had kept the Argentinian navy in port during the Falklands war, he announced that France would add another nuclear submarine to the six ordered since 1984. But Quilès effectively squashed air force hopes to build a new land-based strategic missile, saying that the resources would be better spent on the improvement of submarine missiles. The existing "park" of 18 missiles in Haut-Provence will, however, be retained.

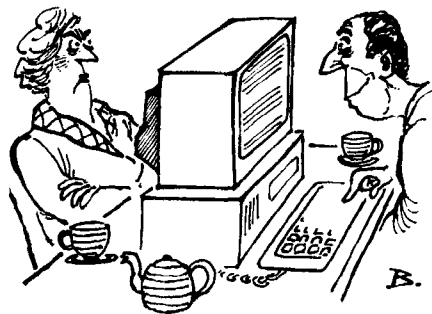
Robert Walgate

Media research

Making the messages make sense

Cambridge, Massachusetts

A 140-MEMBER multidisciplinary team is in the final stages of being welded into a unique research force to wring greater benefits from communications technology at the Massachusetts Institute of Technology (MIT). Among other things, the new \$45 million Media Laboratory will set out to develop "personalized newspapers", which are compilations from some central



database of information selected to match the interests of individuals.

The new project has been seven years in gestation according to its director, Professor Nicholas Negroponte. The idea is to provide a forum for research for members of the MIT faculty interested in the techniques of communication but unable to give their interests free rein within the disciplinary structure of MIT. Now, Negroponte says, there will be creative artists and creative inventors under the same roof. And the Media Laboratory will ensure that the application of a technique is given as much prominence as the development of its technology.

Negroponte has been this way before. Three years ago, he took a year's leave of absence from MIT when President François Mitterrand appointed him as the first executive director of the World Centre for Personal Computation and Human Development, based in Paris. The Media Laboratory will concentrate on eleven areas of research: electronic publishing, learning research, advanced television, computer music, spatial imaging, graphics, the interaction between people and machines, telecommunications, film and video, computer graphics and animation and computer entertainment.

Negroponte emphasizes that many of the projects in these fields now under the wing of the Media Laboratory were begun at MIT as long as 15 years ago. The projects in electronic publishing nicely illustrate what the laboratory is attempting.

One scheme is to develop the techniques for compiling a daily newspaper suited to the needs of different individuals, each of whom would have registered in advance a profile of his interests. Computers would be used to select from some suitable database of the world's news each night items matching the read-

er's declared interests and tastes. The result would be a "personalized" full-colour morning newspaper "inclusive of non-newsworthy but personally important information of the day". In principle, given the development of a suitable telecommunications network, such a newspaper could be delivered to any home or business computer first thing every morning.

There is also a project to compile video cassettes along the same lines. Computers would sift through each day's output of television news broadcasts, selecting items fitting the declared interests of subscribers and, if necessary, editing these automatically so as to leave only highlights.

East European studies

Environmental issues surface

Washington

THE Third World Congress for Soviet and East European Studies two weeks ago revealed a massive interest in problems of environmental protection, health care and industrial planning in the Socialist bloc, taking the organizers by surprise. Although arrangements had been made to accommodate up to 17 parallel sessions, the rooms allotted for the environmental sessions proved consistently too small. In the rest, only subjects of particular topical interest, such as the Ukrainian Catholic Church, drew such crowds.

These world congresses are sponsored by the International Committee for Soviet and East European Studies, an organization which the Soviet academic establishment regards as potentially a source of Western subversion. As at the two previous congresses (Banff, Canada in 1974 and Garmisch-Partenkirchen, West Germany in 1980), a number of Soviet and East European speakers failed to arrive. Although the reasons are not clear, one speaker, Dr Magdalena Sokolowska, from Gdansk, due to talk on the health system in Poland, informed the organizers that she had been refused a passport. Rather surprisingly, however, Dr Ivan Supek, the Yugoslav dissident physicist, did arrive. Supek was prominent in the 1950s in the Yugoslav nuclear research programme, but has since become an anti-nuclear campaigner and Pugwash activist. His participation was all the more remarkable because in 1984 he was deprived of his passport before a planned lecture tour of the United States and Canada (see *Nature* 310, 93; 1984).

The general absence of Soviet and East European scholars, however, and the inevitable predominance in such a meeting-place of Americans, gave a somewhat unfortunate bias to the proceedings.

Papers on West-East technology trans-

The Media Laboratory's projects for the development of high-definition television pictures are more technical. The objective is a fivefold increase of the definition of television pictures compared with present best practice. Hitherto, most research in this direction has involved an increase of the bandwidth of the transmitting medium to accommodate more information for each frame, but the Media Laboratory is investigating the possibility of using computer intelligence at the transmitting and receiving ends of the transmission link. The first step will be to complete the development of a simulator allowing the parameters defining television transmission — aspect ratio, frame rate and the number of scan lines, for example — to be independently variable, so that transmission capacity can be used most economically.

Bill Johnstone

fer tended to dwell almost exclusively on trade between the United States and the Soviet Union, with a number of US presentations pronouncing magisterially on the need for a unified Western stance on strengthening Cocom controls rather than analysing the state of transfer so far and its estimated effect on the Soviet economy.

Several Europeans pointed out the need for greater attention to be paid to technology transfer between Western and Eastern Europe through third (neutral or non-aligned) countries and the special relationship existing between the two Germanies that facilitates both technology transfer and recent joint initiatives in dealing with environmental hazards in the border region. US participants had little to say about this subject.

This US concentration on the Soviet Union entailed an occasional vagueness in dealing with material from the smaller Comecon countries. The British and European papers, on the other hand, tended to be richer in personal and anecdotal detail, such as Dr Fred Singleton's revelation that the Romanian Iron Gates power station, by raising the water level of the Danube, has caused considerable damage to the Djerdap National Park in Yugoslavia, which runs along the river bank, and has also rendered useless land-drainage installations in the autonomous province of Vojvodina.

The US concentration on Soviet (rather than East European) studies, coupled with the lapsing in 1982 of the US-Soviet exchange programme, was undoubtedly responsible for the lack of up-to-date data available to the Americans, and their eager fastening upon unconfirmed reports that the notorious papermill which for a decade has been discharging effluent into Lake Baikal has been, or is about to be, converted to furniture production.

Vera Rich

Endangered species

Japanese flout convention

Tokyo

THE Japanese government is once again under fire for its inability to deal decisively with the illegal import of endangered species.

The problem has been brought back to public attention by a private visit to Japan from Dr Coinbra, director of the Rio de Janeiro Primate Center. Fourteen lion tamarins (*Leontopithecus rosalia*), an extremely rare species close to extinction in the wild, have been illegally imported into Japan and Brazil wants those that still survive to be returned.

Trade in tamarins is prohibited by the Washington Convention on International Trade in Endangered Species of Wild Flora and Fauna to which Japan is a signatory. Unlike other signatories, however, Japan has no domestic legislation to back

up its provision.

What that means in practice is that if an animal can be brought into the country, even by use of false documents, there exists no means to punish the offenders or to force them to return the animal to its country of origin. In the United States, by contrast, under the Endangered Species Act and the Lacey Act, the illegal import of animals can incur confiscation, fines of up to \$20,000 and five years' imprisonment.

The result in Japan is that unscrupulous animal dealers have brought endangered species into the country knowing that if they can get past customs they are home and dry. What motivates them is the big profits to be made in the animal trade. There are plenty of private zoos in Japan whose owners know they can boost their attendance figures by display of rare and exotic animals and seem not too concerned about how they lay their hands on them.

Fourteen lion tamarins were brought into Japan two years ago by using a loophole in the Washington Convention. The convention does permit trade in endangered species that have been bred in captivity. False documents showing the monkeys to have been bred in Guyana were used to obtain an import permit.

There are three races of lion tamarin: golden, golden-haired and golden-rumped, which come from different regions of Brazil. Destruction of the south-east coastal rain forest and its replacement by farmland and the conurbations around Rio de Janeiro and Sao Paulo have reduced their numbers to a few hundred. Logging of the older forests, which provided large trees with holes in which tamarins could shelter, has been particularly harmful. The golden lion tamarin is now the focus of a worldwide breeding programme based at the National Zoo, Washington. A similar programme for the golden-headed lion tamarin is run from the Rio de Janeiro Primate Center and the zoo at Jersey, in the Channel Islands.

The golden-headed race was brought into Japan. Three were re-exported to Hong Kong whose zoo is a member of the international scheme to conserve tamarins. These three have subsequently bred. The eleven remaining in Japan had a less happy fate. One died of stress soon after arrival. Most of the others were transferred to private zoos. Two that were transferred to the Japan Monkey Center at Inuyama were inspected by Dr Coinbra during his visit to Japan. He confirmed what other visitors had found; the two monkeys are kept in a small dark cage indoors where they never see sunlight and receive a poor diet. Judging from their present condition he believed they would not live long. But the Japanese government is

powerless to force their return to Brazil.

That the import of the lion tamarins was allowed in the first place reflects poorly on the Ministry of International Trade and Industry (MITI) which is responsible for granting licences. Anyone with a knowledge of monkeys, or with reference books to hand, would have known that Guyana has no captive breeding programme and that the papers showing their origin must have been faked. MITI's skill in stopping exports does not seem to equal its past skill in promoting exports. Nor are the tamarins an isolated case: 32 tons of ivory were recently illegally brought into Japan from Uganda but it is now too late to do anything about it. And during the recent Tsukuba Expo, some thirty endangered species, including Komodo dragons from Indonesia, were on display near the Expo site.

The closed nature of ministerial dealings does not help matters; in the United States and Europe, applications for import licences may be inspected by interested parties whose zeal is likely to exceed that of government officials. Deceptions are unlikely to succeed.

Despite much public criticism in the mass media, it seems that the Japanese government is making no move towards legislation. The best that might be achieved at present is that unofficial links will develop with conservation groups that will raise the level of the ministry's expertise.

Alun Anderson

Innocents to speak

FRENCH schoolchildren are to be asked what they think of science and of scientists in an inquiry launched by the chief French research council, CNRS, and the educational magazine for 10-15 year-olds, *Okapi*. The fact that the inquiry is taking place is a mark of the degree to which the government plans to forge a new France in which science and technology are the leitmotif for the future.

Okapi claims a circulation of 82,000, and the sponsors of the survey, which will take the form of a questionnaire in a future issue, hope that they will receive replies from a sample of 5,000 French schoolchildren. Among other questions, children will be asked "how confident are you of the progress of science?" and will be expected to answer on a four-point scale ranging from "very" to "not at all".

Many of the questions are concerned with the image of the scientist. Is he a dreamer, or a man of action? (Only the masculine image is offered.) A benefactor or an egoist? Creative or a pedant? A loner or a team-player? But respondents will also be asked to rank countries in order of scientific strength, from a list consisting of Australia, China, France, Japan, Britain, the United States, the Soviet Union and West Germany.

CNRS also hopes to learn from the inquiry something of the way in which French children learn of developments in science. "It's the first time children have been asked to give their opinions on a subject that will affect their future", says one official. But the motivation of the inquiry is also linked with the conviction of the education minister, M. Jean-Pierre Chevènement, that the time has come to break the stranglehold of mathematics and theoretical science in the school curriculum in France.

Robert Walgate

Roast turkey

Washington

SPARKS flew at a recent hearing in Washington on the administration's plans to regulate biotechnology, when Senator Albert Gore quizzed Dr Henry Miller of the Food and Drug Administration on his role in the development of guidelines on biotechnology safety for the Organization for Economic Cooperation and Development (OECD).

Having established that Miller considered himself unbiased on the regulations, Gore produced with a flourish a letter from Miller to Dr Robert McKinney of the National Institutes of Health, in which Miller asked McKinney to review "this turkey" and be "very critical". Miller also asked what "the Rifkins and [Representative John] Dingells will make of this". Miller protested that his meaning had been misunderstood, a reply Gore considered "unacceptable".

The OECD guidelines have had a controversial history (see *Nature* 31 October, p.758) but a draft dated October has now been agreed by US agencies and is to be sent to OECD's headquarters in Paris for review. Some of the more controversial statements in an earlier September draft have been deleted from the final version.

Tim Beardsley

Computer language

When to go back to BASICs

Dartmouth, New Hampshire

BASIC is being debated, says one of its creators, Professor John Kemeny of Dartmouth College. Kemeny, still at Dartmouth's department of mathematics, where BASIC was developed more than twenty years ago, is now calling for a halt to the development of inferior versions of his never-patented language, saying that they are corrupting its purposes and causing chaos in the computer industry as well.

In 1963, Kemeny and his colleague Tho-

mas Kurtz changed the face of computer science forever by inventing BASIC, now probably the most widely used computer language in the world. Their objective was to create a novel learning tool for students. To begin with, they installed a collection of teleprinters linked with a mainframe in one part of a students' recreation room.

The arrival of the microprocessor made sure that BASIC would not be just a Dartmouth teaching aid. Now, after twenty years, Kemeny reckons that there are more than 100 versions of his language, most of them so inferior that he and Kurtz have become seriously concerned.

Kemeny says that the later versions violate the philosophy of the original. If only users would go back to BASICs, educationalists would have a superior tool, while programs written for one microprocessor would be found to work on others. BASIC (which stands for Beginners' All-purpose Symbolic Instruction Code) began, he says, as a device for giving undergraduates simultaneous access to Dartmouth's GE mainframe computers. With the volume production of microprocessors in the 1970s, however, the language began to be used extensively in various forms. Because the memory of the early machines was limited, programmers were at first compelled to improvise, simplifying BASIC and specializing it to fit the memory of small machines.

Kemeny's frustration is that, even though the microprocessors now available do not have the limitations of those being manufactured 20 years ago, the omissions

from BASIC used by the early programmers have not been reinstated. But, Kemeny says, "these machines are not toys, but very powerful computers. The IBM PC I have at home is not a particularly expensive model, but it is more powerful than the original timesharing machine here at Dartmouth." That is why, Kemeny says, many of the BASIC programs now on offer "just don't live up to what any computer scientist would expect of a good language in 1985".

Two years ago, Kemeny, Kurtz and three other computer experts accordingly set up a company to promote the fundamentals of BASIC languages, and to engineer improvements of software developed by other computer scientists. Inevitably, the company is called True Basic.

Kemeny predicted 20 years ago, when opening the computer centre at Dartmouth College, that every home would have a computer terminal within a quarter of a century. The unexpected arrival of the microprocessor has almost made that prediction a reality, but the lack of a common computer language is still an impediment. So one of the goals of the Kemeny group is to develop the language that will make the vision a reality.

So far, the group has developed compilers for the IBM PC and for the Apple Macintosh that will allow them to respond to TRUE BASIC. Two new microprocessors to be launched onto the Christmas market this year will also have such compilers. According to Kemeny, because the compilers written for the individual models embody the necessary changes, programs written in TRUE BASIC for any of these models will run successfully on any other. **Bill Johnstone**

India against AIDS

New Delhi

ALARMED by reports of AIDS (acquired immune deficiency syndrome) in Pakistan, both India and Bangladesh are taking precautionary steps to keep out the AIDS virus. The Indian Council of Medical Research (ICMR) has set up a task force headed by the ICMR chief Dr V. Ramalingaswami. A similar panel under Professor Nurul Islam, director of the Dhaka Institute of Postgraduate Medicine, has been set up in Bangladesh.

No confirmed case of AIDS has been reported either in India or in Bangladesh. But Professor A.N. Malavya of the All-India Institute of Medical Science in New Delhi and a member of the task force says it is only a matter of time. The major aim of the task force is to educate both the doctors and the public about AIDS without creating a scare and to keep tabs on vulnerable groups including haemophiliacs, homosexuals and a significantly large population of eunuchs besides the prostitutes in ports such as Bombay, Calcutta, Madras and Vishakapatnam.

Cases showing AIDS symptoms will be referred to any of the dozen medical institutions to be designated as reference centres. The National Institute of Virology in Pune is being equipped to screen for AIDS antibodies and viruses in blood samples of suspects. The task force is evolving a detailed questionnaire to be circulated to private and government doctors and an educational campaign is planned. But the Indian government does not want to divert huge funds to an anti-AIDS programme as there is no evidence of AIDS yet.

In Bangladesh, the AIDS committee has been asked to prepare a plan of action for the prevention of AIDS. As well as evolving a programme for surveillance, detection and treatment, the committee intends to propagate Islamic ideals on the evils of promiscuity. People have stopped buying imported second-hand clothes following a rumour that they might be contaminated with AIDS virus, and Bangladesh newspapers have asked the government to screen incoming passengers from the West.

K.S. Jayaraman

UK laboratory animals

Debate on legislation ahead

THE British government's Animals (Scientific Procedures) Bill, published last week, will be debated in the House of Lords this month. The bill follows the proposals outlined in two white papers (policy documents), Cmnd 8883 in May 1983 and Cmnd 9521 in May 1985, and is intended to replace the Cruelty to Animals Act of 1876 (see *Nature* 315, 267; 1985).

Attempts to reform existing legislation on laboratory animals have failed in the past because of strong protests from animal rights groups and reluctance of members of parliament (MPs) to become embroiled in the issue. The new bill is supported by the middle-of-the-road animal welfare groups, who advised in drawing it up, but is violently opposed by the extreme animal rights groups who are against any experiments on animals being made legal.

The bill requires that a project licence be obtained before work can begin, con-

trols breeders and suppliers of animals and establishes a committee to provide independent advice to the Home Secretary. All research establishments must appoint a named person to care for animals, there will be heavier penalties for offences and controls on the re-use of animals.

A significant provision is the explicit acknowledgement that pain is caused to animals; licences will be issued only if the Home Secretary is satisfied that the benefits outweigh the adverse effects on the animals and the licence requires that pain, distress and discomfort are minimized.

The bill has wide support from scientists and welfare groups, and its passage through the House of Lords seems assured. But the Commons is very different. MPs have traditionally been extremely reluctant to favour any legislation that makes them seem to be supporting "cruelty" to animals in the eyes of their constituents.

Maxine Clarke

British research councils

Budgets on a shoestring

PROFESSOR Bill Mitchell, new chairman of the largest British research council, the Science and Engineering Research Council (SERC), presented the organization's annual report last week. Sir John Kingman, who was chairman until the end of September, says in the report that the severe financial pressure of recent years has resulted in losses sometimes "out of all proportion to the money saved", particularly in the lack of provision of operating costs for big-money facilities. "The truth of the matter is that the United Kingdom is trying to maintain its science base on the cheap", said Kingman.

Mitchell, taking a more optimistic line than his predecessor, congratulated Kingman on "coping so well in difficult circumstances" but said that the work described in the report was not that of a "demoralised science and engineering community". But like Kingman, he stressed the role of basic research in making discoveries of "economic significance".

One new source of money in the science arena is a joint Ministry of Defence (MoD)/SERC programme. SERC welcomes the influx of money (MoD and SERC will each provide 50 per cent). Projects will be subjected to peer-review in the normal way, and will be restricted to unclassified fields. SERC will assess the scheme next year and "reconsider" if any of its conditions are not met.

The highlight of 1984 was the production of the first neutrons by the Spallation Neutron Source (Rutherford Laboratory, near Oxford) in September. At the Synchrotron Radiation Source (Daresbury Laboratory, near Manchester) the "wiggler" beamline enabled the first enzyme crystal, phosphorylase *b*, to be monitored as it performed its catalytic reaction. Unexpected order-disorder transitions have been found and people are queuing up to use the device which measures 40,000 reflections per second.

In 1984 the Isaac Newton, Jacobus Kapteyn and Carlsberg Meridian Circle telescopes began operation, the Active Magnetron Particle Tracer Explorer satellite was launched and the Anglo-Australian Telescope has allowed the most distant galactic objects yet known to be seen. But the Royal Greenwich Observatory may soon stop participating in the international time determination programme (see *Nature* 31 October, p.754); SERC has decided to support observational astronomy at the expense of other projects.

Engineering science has received increased financial support during the past 10 years so that it now makes up 20 per cent of SERC's budget. SERC is reviewing the effectiveness of its engineering programmes and will decide whether to shift priorities again, or even whether to change its own name (to STRC, T for

technology) in February 1986. Similarly, SERC is reviewing its position on chemistry research, an area that has lost ground in the past three years.

Another controversy caused by government cuts is the question of British membership of the European Organization for Nuclear Research (CERN). Reduced money to SERC has coincided with an increased subscription to CERN and it is likely that some experiments will stop completely, damaging Britain's reputation as a reliable partner in international collaborations. The decision is in the hands of the government, at present considering the recommendations made to it

Soviet plan

More goods, more science

THE "Draft Basic Guidelines" for the Soviet Union's Five-Year Plan (1986-90) are, as usual, not so much a detailed economic programme as a manifesto eulogizing the Soviet way of economic life. The preamble extols the progress in the "scientific and technical revolution" during the past five years. It says that nuclear power now accounts for one tenth of all Soviet generating capacity and that the Soviet Union, "which earlier took first place in the world in terms of oil extraction", now holds a similar position with regard to natural gas. Free textbooks are now provided in schools, the housing problem is "being consistently solved" and the natural wealth of the eastern and northern regions of the Soviet Union are being opened up at an "accelerated rate".

The guidelines for the future present a somewhat less happy picture. The "supreme goal" in the next five years and for the remainder of the century is the "steady upsurge of the standard of living" of the Soviet people by raising the efficiency of production. In pursuit of this aim, the food programme (Brezhnev's last legacy to the Soviet people) is to be implemented to ensure the "scientifically substantiated norms of rational consumption", while there is a package of measures to improve the health service. There have been complaints in the Soviet media about poor performance in the health service, perhaps because the energetic anti-alcohol campaign led to the neglect of other aspects of health.

The emphasis on the citizen-consumer reveals, perhaps, how much this sector has been neglected in previous plans. Production of non-food consumer goods is to be increased by more than 80-90 per cent, and the "volume of services" delivered will, according to the forecasts, be more than doubled. But other targets, too, are set equally high — gas extraction at 60-80 per cent more than in the current quin-

quennium and nuclear power increasing between five and sevenfold. In industry, there is to be a general inventory of "fixed production capital", and more than one third of it renewed. The opening up of Siberia and the far east to industry is to continue, although there is now increased emphasis on environmental protection.

In science, the role of the Soviet Academy of Sciences as co-ordinator of research is to be "enhanced", as also will be the role of the academies of the union republics. The draft plan speaks of utilizing the scientific potential of the higher education system, and expanding the research and development work in the universities. There is to be closer interaction between the research facilities of the academies, the universities and the specialized institutes of the production sector, and "progressive new forms" of research organization will be sought.

Several scientific fields are listed for special development, including theoretical and applied mathematics, information science, cybernetics, particle, nuclear and solid-state physics, micro and quantum electronics and also research into nuclear and non-traditional energy sources. The "scientific principles" of catalysis, chemical technology and biotechnology are to be "elaborated" and the mass production of personal computers is to be "organized". The supply of modern instruments, materials and other necessities to research facilities will be "substantially improved".

The guidelines are nevertheless unclear about the relative roles of pure and applied science, saying that the "fundamental task" of the current plan is to "strengthen ties between science and production", while, at the same time, "priority significance" is to be given to fundamental research which "predetermines the attainment by social production of a qualitatively higher level". **Vera Rich**

Foreigners' tenure at Tsukuba

SIR—I should like to add a gloss to Alun Anderson's fair report on the "turmoil over treatment of foreign staff" (*Nature* 10 October, p.465) at Tsukuba University, self-proclaimed champion of the internationalization of university education in Japan.

Four foreign staff members, including myself, expected to be given long-term contracts not because "some supporters made affirmative responses" to them, as Tsukuba's president, Nobiyuki Fukuda, says, but because the due process for their appointments had been put in train: the vice-president in charge of education, *ex officio* member of the personnel committee that makes final decisions in personnel matters, first ascertained the need for our posts at the departmental level, then discussed his proposal to give us long-term contracts at a meeting of the president and the five vice-presidents, none of whom voiced any opposition. Thereupon he instructed the department heads to let us know that we were scheduled to get tenure. The department heads prepared the formal applications to be submitted to the personnel committee. I therefore think that our expectations were not "extremely strange" as Mr Fukuda believes, but only reasonable. What is strange is that the final authority on personnel matters was not given a chance to make its decision as neither the departmental applications nor the supporting proposition of the vice-president were considered by a meeting of the personnel committee — apparently both were blocked when the vice-president was sacked.

While it is true that there is no final definition of "internationalization", I think there is agreement on certain ingredients that it must contain, such as non-discrimination on grounds of nationality, transparency and minimum civility. Tsukuba University is still in want of all of them, I am afraid. Anderson amply illustrated that foreigners at Tsukuba are not treated "just like anyone else". It might be added that Tsukuba did not avail itself of the opportunity the new law offers to give tenure to foreigners. For foreigners at Tsukuba, "tenure" means just 5-year contracts, while every Japanese staff member gets real tenure from the start. Foreign lecturers have to carry a teaching load of 12 hours per week whereas their Japanese colleagues average five.

The rule limiting employment of foreigners without tenure to four years resulted from the unwillingness of university authorities to speak their mind clearly. Not all foreign lecturers have always performed their task satisfactorily. The university therefore introduced a system grading each foreigner (but no Japanese) each year as A, B or C. Yet rather than tell the Cs to improve or leave, all lecturers had to quit, the As along with the Cs. The

announcement was made in writing, with no explanation.

"Inexplicable silence followed by brazen behaviour", a Japanese attitude regarded by many Westerners and Japanese as a serious obstacle to internationalization, also characterized the recent events at Tsukuba that Anderson described. No wonder then that Tsukuba is seen as becoming more parochial rather than internationalized. To dismiss this view as "nonsense", as Mr Fukuda does, does little to solve the problem.

MARGARETE SAWADA

Namiki 3-518, Sakuramura,
Ibaraki 305, Japan

Reputable PhDs

SIR—"A PhD is supposed to be a training in research" says Beverly Halstead (*Nature* 316, 760; 1985) and therein lies his problem. I suspect that some proportion of every thesis (for example the research training part) will not meet the standards of the existing avenues for publication. That is not to say that some of this proportion would not be useful to someone embarking in that particular area of research. Thus there exists a case for making theses, in their entirety, more readily available globally through some other means (for example microfiche).

Attempting to distinguish major from non-major contributions to science would be headache enough, but differentiating between reputable and non-reputable journals is a job I leave for Beverly Halstead. I look forward to his published list appearing in these columns.

JONATHAN A. KEOGH

Department of Botany,
University of Botany,
University of Otago,
Dunedin
New Zealand

Units are for people

SIR—Although no system is proof against mistakes, spectacular events, such as the apparent misprogramming of a space-shuttle computer (*Nature* 315, 702; 1985), highlight the usefulness of an agreed universal system of units. D.C. Jolly (*Nature* 316, 480; 1985) enters an impassioned case for the foot, pound, second system, as an alternative to SI and pleads for a counter-revolution. W.G. Rees (*Nature* 317, 10; 1985) has suggested an ingenious system which differs from the foot, pound, second system by a possibly tolerable amount, such that c , G and $4h$ become exact multiples of his base units.

Unfortunately, his suggestion is impractical since, for example, we cannot yet measure all of the fundamental constants and atomic phenomena with the required accuracy. Indeed, as we do so, the SI units

too will incorporate the fact, for the Conférences Générales des Poids et Mesures will revise the recommended definitions of the base units accordingly, as they have with the second and metre — but without altering their size.

While it is tempting to argue for a change to a "natural" system of units, we ought to recall that part of the appeal of the c.g.s. system to our nineteenth century forebears stemmed from its use of "natural" quantities associated with our planet. Who can be sure that the fundamental constants e, c, h , G and so on will not have been replaced by an ever deeper set by the end of the next century? Moreover, a system only appears natural if the units are appropriate to the discipline with which the proposer is most familiar — what is "natural" for the physicist is not necessarily so for the biologist, chemist, artisan or "man in the street".

We might yet, if we chose, be able to recycle some of our earlier units instead, for G is near enough unity in the Gunter link, troy grain and lunar month system of units, and the Planck constant essentially 1 attoleague², megascruple, centiweek⁻¹ (British nautical leagues, apothecaries scruples and sidereal weeks). Professor Kenneth Meyer, at the University of Cincinnati, has shown how unit conversion tables (in the *Handbook of Chemistry and Physics* and elsewhere) together with a microcomputer allow such combinations to be found — even an exact one by an undetectable massaging of the magnitude of the earlier unit! I suggest though that readers wait for the forthcoming CODATA evaluation of the recommended "best values" of the fundamental physical constants before searching for the best such combination of earlier units to six decimal places!

B.W. PETLEY

National Physical Laboratory
Teddington,
Middlesex TW11 0LW, UK

Sir Henry Wellcome

SIR—In the article on the Wellcome Trust and Foundation (*Nature* 24 October, p.662), you refer to them as being set up under the will of Sir Henry Dale. I believe that it was Sir Henry Wellcome who in his will created these institutions.

As a young prescriptions clerk in Rochester, Minnesota, Wellcome was encouraged and supported by Dr W.W. Mayo to improve his education and not to be satisfied with his position. Mayo had been taught chemistry by John Dalton before emigrating to the United States. His sons, William and Charles Mayo, created the Mayo Clinic.

LARS BREIMER

Department of Chemical Pathology,
Royal Postgraduate Medical School,
Hammersmith Hospital,
Ducane Road,
London W12 0HS, UK

Evolution and education

SIR—Your erroneously titled "Californian setback for evolutionists" (T. Beardsley, *Nature* 5 September, p.3) described an unprecedented recommendation by California's Curriculum Commission: that no science textbooks be adopted for grades 7 and 8 unless the books be revised to give proper treatment to evolution and human reproduction, among other subjects. This was a significant defeat for creationists and others who try to subvert science education in this state. The defeat was certified on 13 September, when the Board of Education accepted unanimously the Curriculum Commission's plan.

Beardsley erred when he attributed to California an "antidogmatism law". No such law exists. But the Board of Education has an antidogmatism policy, which dictates: "That, on the subject of discussing origins of life and Earth in public schools: (1) Dogmatism be changed to conditional statements where speculation is offered as explanation for origins. (2) Science should emphasize 'how' and not 'ultimate cause' for origins."

This grotesque declaration, adopted in 1972, is noteworthy for many reasons. First, it is blatantly dogmatic. Second, it can be explained only as a device for appeasing creationists and for abetting their attempts to distort the teaching of science. Third, it erects a wholly specious, intellectually indefensible demarcation between discussions of "origins of life and Earth" and discussions of all the other subjects that science addresses. Fourth, nobody can say precisely what the policy means or requires, because none of its terms — dogmatism or conditional statements or speculation, for example — is defined. Fifth, in an antic, confused decision rendered in 1981, a judge of California's Superior Court suggested that the policy should apply not only to discussions of "origins of life and Earth" but also to discussions of organic evolution. Sixth, since 1981, creationists in California have relentlessly and conspicuously invoked the policy (and their own interpretations of its surrealistic language) during efforts to vitiate or suppress discussions of evolution in textbooks and in science classes. Seventh, because the policy's only evident purpose is to serve the adherents of a particular religious dogma, the policy's constitutionality is (to say the least) questionable.

WILLIAM J. BENNETTA
PO Box 26603,
San Francisco, California 94126, USA

Tim Beardsley writes: In the 1981 case referred to by Bennetta, Sacramento Superior court Judge Perluss not only upheld the validity of the antidogmatism policy but also ordered the State Board of Education to disseminate it to all interested parties; the policy may therefore be not entirely without legal force. □

Teenage SCOPE

SIR—Your article entitled "What to make of nuclear winter" (*Nature* 19 September, p.189), mentions "the working party called the Scientific Committee on Problems of the environment (SCOPE) set up three years ago by the International Council of Scientific Unions (ICSU)". This sentence give a wrong picture of our committee. As you might know, SCOPE is one of the ten scientific committees of ICSU and was established in 1969 to assemble, review and assess the information available on man-made environmental changes and the effects of these changes on man.

In 1982, the SCOPE General Assembly decided, at the request of ICSU, to appraise the state of knowledge of the possible global environmental impacts of nuclear war. That project, called ENUWAR, may have been the most important of some ten international scientific projects undertaken by SCOPE during the past triennium, but it does not overshadow the contribution of SCOPE during the past fifteen years to the advancement of scientific knowledge on many problems of global environmental concern.

VÉRONIQUE PLOCO
(Executive Secretary)

SCOPE,
51 bd de Montmorency,
75016 Paris,
France

Velikovsky's evidence?

SIR—Lynn Rose's defence of his interpretation of the Venus Tablets¹ may be seductive, but his conclusion is neither as inevitable nor as secure as he argues. He states that the tablets "strongly support Velikovsky's theory that Earth has undergone catastrophic orbital change within historic times". However, there is no explicit connection between the tablets and Velikovsky's catastrophes. Indeed, the change implied in the shape of Earth's orbit is a far cry from the intersecting orbits discussed by Velikovsky. The only philosophically justifiable conclusion is that Rose's interpretation of the tablets "is consistent with" Velikovsky's theory, not "strongly supportive".

In contrast to Rose's insistence on ignoring as few of the observations in the tablets as absolutely necessary, he argues his case favouring Velikovsky while ignoring a plethora of contradictory data. The falsification of Velikovsky's scenario provided by Greenland's Dye 3 ice core² is corroborated by ocean sediments and bristlecone pine rings³ and the revised late-glacial Swedish varve chronology⁴. Either their mere existence contradicts Velikovsky or they do not contain debris suggesting a catastrophe. Velikovskian catastrophes are neither indicated by nor necessary to explain the natural stratigraphies of the Holocene.

Furthermore, according to Rose's own

work with Vaughan⁵, the sequence of orbital changes implied by *Worlds in Collision* that conserves angular momentum without increasing orbital energy starts with the untenable condition of Earth closer to the Sun than Venus now is. Surely such a severe physical constraint⁶ is to be accorded more weight in assessing the validity of Velikovsky's theory than an idiosyncratic interpretation of the enigmatic Venus Tablets.

The Terminal Cretaceous Event 65 million years ago, whatever it was, left unambiguous, worldwide signatures of iridium⁷ and soot⁸. The catastrophe Velikovsky conjectured within the past 3,500 years left no similar signatures. In this light, advocating the validity of Velikovsky's scenario, as Rose does, strains the bounds of credulity, both scientific and philosophical.

If the Venus Tablets preserve authentic observations, however disguised by copying errors, then explaining the discordant observations needs to be accomplished with new insight. For example, John D. Weir has suggested to me that if a formerly very bright comet periodically altered the sky's brightness, then the dates of appearances and disappearances for Venus would be affected. The debris from the disintegration of comet Encke 4,700 years ago, discussed by Clube and Napier⁹, would be a possible agent. The clay of the Venus Tablets is far too fragile a foundation upon which to justify a new physics for the rationalization of Velikovsky's theory.

C. LEROY ELLENBERGER
3929A Utah Street,
St Louis,
Missouri 63116, USA

1. Rose, L. E. *Nature* **317**, 470 (1985).
2. Ellenberger, C. L. *Nature* **316**, 386 (1985).
3. Ellenberger, C. L. *Kronos* **X:1**, 92-97 (1984).
4. Cato, I. *Boreas* **14**, 117-122 (1985).
5. Rose, L. E. & Vaughan, R. C. *Pensee, Velikovsky Reconsidered*, 110-132 (New York & London, 1976).
6. Ellenberger, C. L. *Kronos* **X:1**, 91-92 (1984).
7. Alvarez, L. W. *et al. Science* **208**, 1095-1108 (1980).
8. Wolbach, W. S. *et al. Science* **230**, 167-170 (1985).
9. Clube, V. & Napier, B. *The Cosmic Serpent*, 150-153 (New York & London, 1982).

Help wanted

SIR—In collaboration with Professor Richard Gregory, Professor Dorothy Hodgkin, Sir Fred Hoyle, Sir Peter Medawar and Dr Jonathan Miller, I am preparing an anthology of science writing, to be published by Basil Blackwell. The intention is to bring together a wide variety of pieces, ancient and modern, of varying length, and drawn from all sectors of science, the sole criterion being that of literary excellence. We would be delighted to receive suggestions from readers of *Nature* regarding items suitable for inclusion in what we hope will prove to be a work of sheer aesthetic and intellectual delight.

BERNARD DIXON
81 Falmouth Road,
Chelmsford, Essex CM1 5JA, UK

Industrial power by research?

China's science is in the process of being harnessed to ambitious social and economic goals. This survey of science in China may suggest whether the venture will succeed.

Will China make it? And how soon? Or will the present still-short period in China's recent history prove to be another aberration, like the Great Leap Forward (1960–66) or the Cultural Revolution (1966–76)?

In China now, opinion is unanimous. The largest nation on the Earth is at last on the road to becoming an industrial power matching in prosperity and creativity the most successful nations elsewhere in the world. The job will be done by adapting science and technology to China's distinctive talents, of which the Chinese are self-conscious and proud. The task, of course, will take time — half a century or even more — but what is that by the yardstick of China's three millennia of recorded history?

The optimism is almost palpable — and is the only credible explanation of people's apparent willingness to endure a degree of personal deprivation, material and otherwise, that still shocks people from the West.

This survey of science in China is inevitably incomplete, less a survey in the strict sense than a reconnaissance. The government of China claims that there are more than 4,000 independent research organizations in China, which, if true (there are problems of definition), implies that the account that follows is based on a one per cent sample which is, moreover, biased towards institutions whose reputations travel.

Even so, there are good reasons to believe that important generalizations can be safely made. First, there is the curious phenomenon, entirely unfamiliar in the West, that government officials are the first to agree that there are obvious shortcomings in their organization for China's objectives; often, they will go on to explain what they plan to do about these problems. Second, there is the uniformity of the common conviction that the ship is sailing in the right direction: it would be churlish to ask whether China has always been so certain of itself.

China is also a Marxist state, in this as in many other respects the largest anywhere. Those who may think that the political system must surely be irrelevant to a description of what laboratories are like and to an appraisal of how the system is orga-

nized are profoundly mistaken. The truth, in China, is that communist principles and organizations influence everything, not only the distribution of power between the Party and the government but the conditions under which ordinary people live and researchers go about laboratory work. China's goal is not merely to become an industrial power of the first rank, but to remain a communist state.

For the time being, the disciplines and controls stemming from the party's influence, not to mention the way in which the patriotism engendered by the present optimism is strengthened by the party's control of press and television, may help to keep China moving forward single-mindedly. It is an absorbing but unanswered question whether, somewhere along the road, intolerable contradictions will arise. Meanwhile, it is best to remember that the break with the Soviet Union in the late 1950s came about because the then Chinese leadership believed itself alone to be following the true precepts of Marx.

Enthusiasm

Much of the enthusiasm of the present nevertheless springs directly from the way in which the system that gave rise to the Cultural Revolution is now being changed; "reform" is the slogan. The reopening of China to the outside world is one sign of the change; China has gained a torrent of tourists, and is now looking for the generous transfer of technology from the West. The decentralization of the power to transfer administrative (but not political) decisions to provincial governments and operating agencies of the government is another; efficiency is the goal. An uncovenanted side-effect may be that able people no longer feel that only the local general secretary of the party has real power.

The most daring of the reforms so far has been the much-publicized decision that peasants may sell their produce on free markets wherever they have access to them. One result has been a sharp increase of agricultural production (to which other influences have contributed), another is the persisting argument between the party purists and pragmatists about the propriety (and wisdom) of letting loose the genie of the search for gain.

Yet for now, the economic consequences of reform are its sufficient justification. During the past five years, agricultural and industrial production together have increased at a rate of more than 10 per cent a year, too fast for comfort. So the new five-year plan, presented in outline to a National Party Conference in September, will be based on the assumption that growth in future will be moderated to between 7 and 8 per cent a year, just about enough to meet the objective of the Party Congress in 1982 that there should be a fourfold increase of production by the end of the century.

Enterprise

The temptation to attribute these changes to the mild breath of free enterprise in the economy is strong, but would be mistaken. Obviously, economic motives have helped, but only marginally. Eighty per cent of the population of China still lives on the land, and only a tiny fraction of these people, those who by chance live near cities allowing the import of produce other than through the state purchasing agencies, are able to benefit.

The countryside has become more productive for two other reasons, the improvement of crop strains in the past few years (and their general and rapid adoption under the party's influ-

Explanation of a map

To many in China, the choice of an eighteenth-century map, however interesting, to decorate the cover of this special issue of *Nature*, will seem inappropriate. Why not a rocket taking off, or a power station, or a textile mill or something else that will represent the modernity of emerging China? The choice, deliberate as it is, is intended to be symbolic in several different ways.

First, everybody visiting China must be aware of China's sense of its own antiquity, from which may derive its people's confidence, fatalism and patience; this map puts China at the centre and the rest of the world around the edges.

Second, the map shows China as a kind of island which, in spite of the opening to the outside world in the past eight years, China still is. The changes of the past few years have been dramatic, but China is so huge that its contacts with the outside world remain geographically peripheral; the special economic zones are the modern equivalents of the coastal concessions of the eighteenth and nineteenth centuries. In the cities, foreigners are lavished with hospitality but are otherwise still mostly isolated in the foreigners' hotels from which occasionally they venture to spend foreign currency in the shops set aside for foreigners. □

ence), and the concomitant rapid growth of rural industries (which is China's intended but still untested defence against the usual flight from rural to urban slums). In other words, the science of plant-breeding has already contributed substantially to prosperity.

The same is true in manufacturing industry, but to an unquantifiable extent. Indigenous capital investment in new factories has certainly played an important part. So too have the countless joint ventures with overseas high-technology companies which, by creating enterprises for the indigenous manufacture of modern equipment such as telephones, allow the value of China's output to be increased without a proportional investment of indigenous skill. (McDonnell Douglas, for example, will soon start shipping to Shanghai kits from which civil aircraft will be assembled.) Vast as China is, it seems generally and realistically to be accepted that it will be a long time before the country's technical community can sustain the government's industrial ambitions.

Impediments

Several obvious impediments are listed in what follows, but in a spirit that needs careful definition. China, by its own assessment, is a developing country that has somehow survived two centuries of foreign occupation and civil war to enjoy less than a decade of single-minded social and economic progress. The most remarkable feature of the present state of affairs is that China's ambitions cannot be laughed out of court. Another is that the government, whatever its political character, appears to have a realistic appreciation of what the difficulties will be.

The most serious obstacle to continuing change is the shortage of technical skill. In university departments and research laboratories, the lack of technicians means that creative people spend much of their time on laboratory chores. Nowhere are there the small armies of people needed to make headway quickly in fields where numbers matter — writing software for digital telephone switching systems, for example.

Yet it is only within the past few years that, on the academic side, China has had the confidence to mount its own PhD programmes in universities and research institutes. In applied research, the most glaring shortage is of people competent to mount research and development programmes within the framework of industrial enterprises. The most acute shortages appear to crop up in the medium-sized enterprises which, elsewhere, are the chief agents of technical change.

The government acknowledges the difficulty. The next five-year plan will see an increase of recruitment to the universities (by a modest 20 per cent to an annual intake of 500,000 students), but the chief emphasis will be on the training of technicians, in formal technical colleges and in vocational schools linked with the factories. If there is to be an expansion of the university system that would give China a participation rate comparable with that of say Britain (let alone the United States or Japan), that will be postponed to a later plan.

Often, it emerges in conversation (see p.219, Beijing polytechnic) that there are influential people who think China does not need to follow the models established elsewhere; if the enterprise is properly planned (as communist ideology says it should be), a single research institute can take responsibility for the development carried out in the West by several competing enterprises, so that technical skill will be used more efficiently. There are the makings of future rows in this difference of opinion, but not yet.

Difficulties

A much more serious difficulty is the way the research enterprise is organized. China does not, at present, benefit as it might from the skills at its disposal. China's public institutions and enterprises — until recently, there were no private enterprises — are organized along the lines now familiar in the Soviet Union. At the very top, government ministries more often correspond to what would be called executive agencies in the West (which explains why, in China as in the Soviet Union,

there are so many of them). Their job is not so much to make policy that others follow, but to ensure that predefined tasks are executed. So a hypothetical ministry of electric power will not merely build and operate electricity generating stations but also build factories in which to manufacture generator sets and the other equipment needed. To ensure a supply of trained people, it will found a university or college at which promising young people can be trained, and it will then undertake to provide those whom it employs with housing and their children with schooling. A ministry or other large public organization is like a state within a state, commanding the undivided loyalty of its employees, but bound to them by the duty to provide the necessities of life.

Revolutions against previous feudal systems have plainly been less radical than their sponsors have imagined. In the Chinese Academy of Sciences and its institutes and at other academic institutions, the paternalism of the institution towards those whom it employs is taken for granted by both parties. Specifically, those graduating from universities will be assigned to jobs in commerce or industry within quotas provided by the government, but may also apply for posts at research institutes. If successful, the corresponding quota slot remains unfilled, and the student stands on the lowest rung of the research ladder which may, with further success, offer the chance of a PhD course, perhaps overseas, or even a fellowship somewhere else. Promotion within the institute is the rule; sometimes, a transfer to an administrative post with the parent organization is possible. Salaries are poor but costs are low; people pay a few yuan a month (\$1=2 yuan) for the apartments with which they are provided. At suburban research institutes, there will be communal facilities such as a pond stocked with carp. The University of Beijing runs no fewer than three schools for the education of the children of the faculty.

Benefits

The benefits of such arrangements are plain; young people joining a research institute know that they must expect to succeed, at least in the first instance, within that framework. They are accordingly loyal, diligent and respectful. But if, with the passage of time, they should be less than wholly successful, there is no incentive for them to move elsewhere, and every incentive for them to stay. Research institutes become families, greatly extended families. The managers of the system now recognize that the consequence is often stultifying immobility, and several attempts to tackle the difficulty are under way — the secondment of people to factories without loss of academic status, limited postdoctoral fellowships and the like. But the effects of these innovations will be only marginal while the economic system depends as much on rewards in kind.

The doctrine of the self-sufficient public agency is another impediment to the effectiveness of the professional research cadres. Even if it had been possible to buy liquid nitrogen and

Chinese names confuse

THE romanization of Chinese characters is a problem and in an effort at least to standardize and simplify it the Chinese government adopted in 1979 *pinyin* as the official romanization system. The *pinyin* system underpins the drive to establish a nationally-recognized spoken language. This system has been followed as far as possible in what follows. Certain spellings such as Beijing for Peking are now becoming familiar but Guangzhou for Canton perhaps less so. Shanghai remains the same, but the Yangtse river which enters the sea at Shanghai is now written Changjiang. The structure of Chinese personal names presents additional problems but two-syllable (two-character) components are often now written as a single word. Two of the most famous names, Mao Tse-tung and Chou En-lai, are now rendered Mao Zedong and Zhou Enlai. Hong Kong, which has some claims to retain this spelling at least for the present, has not been referred to as Xianggang, the *pinyin* romanization. □

other cryogenic gases on the industrial market, the suspicion must remain that the Chinese Academy of Sciences would have preferred to make its own, for fear otherwise of having to depend on other agencies, with different goals, for strategically important materials. These traditions of self-sufficiency have some advantages, not least in providing working scientists with a sense of the economic cost of laboratory supplies that might otherwise be taken for granted. But the diversion of creative people's time and energy to the simplest of laboratory tasks is a serious waste. Chinese who have spent time overseas are constantly remarking that their counterparts elsewhere can order supplies by telephone, and often expect them to be delivered the following day. Often, it seems easier for a Chinese laboratory to order an expensive piece of equipment from a foreign company (because the provision of funds for advanced equipment has been generous almost to a fault) than to spend a fraction of the cost buying goods and services within China.

The ever-present influence of the Communist Party is, on balance, another brake on effectiveness. But the issue is neither black and white nor immediate, as it may seem, from the West, it is bound to be. China's traditional respect for scholarship has ensured that the heads of institutions are invariably able people. The notion that for every such person there should be a party secretary, usually full-time, whose constituency consists in principle of the whole institution, but whose criteria for deciding what should be done may be qualitatively different, would seem a recipe for continual disaster. The Cultural Revolution shows that disasters on a national scale can happen. But for the present, the chief influence of the institutional party seems generally supportive of what the managers seek to do. So long as the cry is that science must do more to make China's industry prosperous, and while research directors share that aim, there is unlikely to be trouble. It is naturally impossible to tell how often original lines of enquiry are stifled by this unfamiliar consensus.

Survival

Against this background, perhaps the most remarkable feature of the research enterprise in China is that it has somehow been able to survive. Where else could half of a country's research workers have been compelled to work as manual labourers (or worse, imprisoned or simply killed) for between four and six years and then be able to return to pick up the threads of their old projects? It may be true that much of the research at China's research institutes and university departments consists of an attempt to replicate what has already been done elsewhere, but it also often has a distinctively Chinese flavour. People are anxious to learn other people's techniques, but the problems they intend to solve are their own. The success, at a host of China's laboratories, in identifying the active principles of traditional Chinese medicines, and the way in which the Institute of Genetics at Beijing has used the techniques of meristem culture to improve and propagate crop species not tackled elsewhere, should be a proof of that.

In any case, if shortcomings persist, they are now to be removed by means of the reforms decreed in March (see p. 222). That, at least, is what the officials in Beijing would like to think. In future, researchers will be able to succeed either by winning grants for the support of their research from a National Science Foundation still to be set up or by engaging in some kind of industrial activity. (Contract work for industrial enterprises will be commonplace; the search for innovations with industrial potential will be the more daring course, but sometimes people will turn their laboratories into factories.) Those who do not succeed in either course will keep their posts, their salaries and their apartments, but will be subjected to social pressure to do something, which is one of the ways in which the Party may help. So will this not powerfully contribute to China's goals?

Yes, but also perhaps no. That the new arrangements will increase the interest of researchers in China's industry is certain, but there is at least some cause to fear that China's industry may be so short of technicians and technically trained managers that the intervention of more researchers from academic institutions

may be an embarrassment. As things are, the proportion of young people entering higher education is about three per cent. Nobody will be surprised that there are too few engineers, and it is wise that the next five-year plan (to begin in January) should allow for more expansion of technical than of university education. But it will also be a surprise if, ten years or so from now, the then Chinese government is not bewailing the folly of its predecessors in neglecting the rapid expansion of higher education. China has done wonders in the past three decades, by making more than 90 per cent of its population literate; should it now adopt the goal of bringing out the full potential of that literate population? The government may have underestimated the scale on which higher education needs to grow.

Research

It may also have underestimated the importance of basic research to developing China. To be sure, the government is busy with high-energy physics and with space research. But much of China's interest to the outside world, and capacity to win its collaboration, rests on the high quality and the distinctive flavour of what is best in China's research laboratories. Natural cleverness helps. The tradition which China's science has recently cultivated of setting itself impossible goals and then attaining them (the synthesis of insulin is the best-known example) is potentially stimulating for the rest of us.

There are several jokers in this pack of cards. The contrast between mainland China and Hong Kong is not the consequence of an historical accident, but is a sign of what difficulties the slowly waking mediaeval state will have to face in a very few years. The mainland Chinese who now comply with regulations about where they may choose to live, or how many children they may have, are the same Chinese who have made a scattering of improbable islands into one of the richest manufactories in the world. When the mainland Marxists shake their heads over the objective realities of the Marxist dogma, they are talking theoretically; what will they do faced with the contrast between Chinese peoples' inclinations and their system?

Overseas relations are also important. China for the past decade has revered the United States and, by extension, the rest of the West. Now there is a grudging recognition that Japan is passably a success. In the end, it seems more probable that China will turn eastwards, not just to Japan but to the Pacific as a whole. But what if it made common cause with India, another continent from which it is divided by its common character — a huge and diverse population in the fertile sub-tropics, a gulf between the peasants and the rest, a sense of antiquity?

So how should the outside world respond to what is happening in China? The urgent need for training for young scientists has been acknowledged and partly met, but more generosity would be seemly, even prudent. A greater interest in what is happening in Chinese science would be worthwhile in itself, but would also help to strengthen China's self-respect. Too many of China's journals go unread. More visitors not so afflicted by culture-shock that they can say what is wrong in what they see would be welcome, if only as a mark of the adulthood of China's science.

For China, the immediate problems are economic and political. The need not to grow more than twice as fast as the rest of the world seems to have been appreciated. Whether China will be able to achieve the goal of being as prosperous as the West while retaining its communist ideals is both an economic question (what kinds of incentives will be how generally spread?) and, obviously, a political question. Technically, and by its talent, China's research community could easily provide what the government expects of it. Not only China's technical community, but the rest of us, have a vital interest in knowing how the economic and political questions are resolved.

This survey has been written by Alun Anderson and John Maddox, whose visits to China in July and September respectively were arranged by the Chinese Academy of Sciences (Academia Sinica). *Nature* is grateful to the academy, and to the directors and staffs of many of its institutes for their help. □

How China runs its science

A long chain of command

At the very top of the pyramid of organizations involved in science and technology comes the **Science and Technology Leading Group of the State Council**; essentially a group of members of the State Council with a special interest in science and the power to push important measures through. The State Council is the highest body concerned with the day-to-day running of the nation — it is the equivalent of the Cabinet in a Western country and is supervised by the Standing Committee of the National People's Congress. Members of the National People's Congress — the highest representative body — are elected every five years and in turn elect the Standing Committee and the Head of State, currently Li Xiannian.

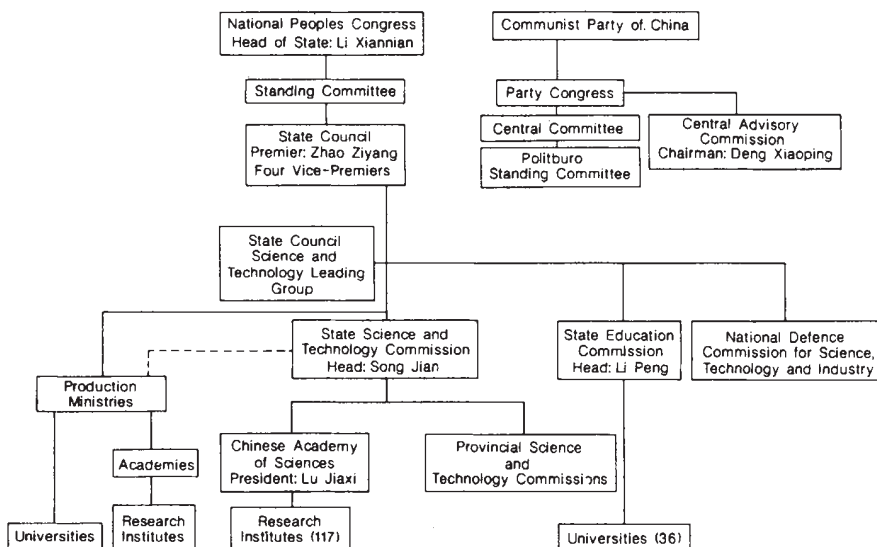
Although the State Council supervises all the ministries and agencies, as well as the People's Liberation Army through the Ministry of National Defence, it does not lay down fundamental policy. That job lies with the largest political party, the Communist Party of China (CPC), and in particular with the Standing Committee of the Politburo of the CPC. Almost 40 million people are members of the party, membership requiring, among other things, a willingness to dedicate a great deal of one's spare time to Party work. But the CPC is not China's only party. Eight others exist although they lack any real power.

Members of the State Council are frequently also members of the Standing Committee of the Politburo and, of course, most of those elected to the National People's Congress are members of the CPC. Not all Politburo members hold state office; Deng Xiaoping, the most powerful man in China, does not.

Immediately under the State Council comes the **State Science and Technology Commission (SSTC)** with responsibility for formulating and implementing science and technology policy within the guidelines given by the State Council. Overall planning and financial support are worked out in cooperation with the State Planning Commission and the State Economic Commission.

Under SSTC supervision but with a great measure of independence comes the **Chinese Academy of Sciences**, whose 117 research institutes carry out the bulk of China's basic research and are well known to academic visitors from the West.

Also under direct control of the State Council are the various ministries. One, the Ministry of Education, became the **State Education Commission (SEC)** in June of this year, a jump in status which reflects important policy decisions on education. Attached to the SEC are 36 universities including many of the most



prestigious "key" universities. But there are many universities which do not belong to the SEC but are attached to individual production ministries, for example, the Ministry of Petroleum has five universities.

In total, the number of ministries is very large by Western standards. Even though reforms in 1983 halved the number of high-level government bodies, there are still 33 ministries and 6 commissions (as against just 12 ministries in Japan). Just why there are so many is easy to understand when it is remembered that all the means of production are owned by the state. To administer and plan their activities a whole set of industrial ministries is required: aviation, chemicals, coal, machine building, metallurgical industry, nuclear industry, space, textiles and so on, just as in the Soviet Union. Many of these ministries have their own schools as well as universities; they may have their own farms and own factories supplying consumer goods for their workers. Each ministry is thus semi-autonomous.

Some ministries also have their own research academies; best known are the **Chinese Academy of Medicine** under the Ministry of Public Health and the **Chinese**

Academy of Agricultural Sciences under the Ministry of Agriculture, Animal Husbandry and Fishery. Each academy in turn runs several research institutes. Individual factories and state enterprises supervised by the various ministries also maintain research laboratories, some of them large.

At the provincial and municipal level of government, much of the above pattern is repeated on a smaller scale. A **Regional State Science and Technology Commission** coordinates local research efforts and bureaus of the local government which match the central ministries look after local industry and solve their technical problems through small research institutes.

Almost all basic research is carried out by the big national institutes: the Chinese Academy of Sciences, the "key" universities, and the academies belonging to ministries.

The large number of scientific societies in China is coordinated by the **China Association of Science and Technology (CAST)**, an organization of growing importance not only in arranging academic meetings and international exchange but in its educational activities and in the expert advice it gives to state bodies. □

The new reforms

Pragmatism now the watchword

DRAMATIC changes are taking place in the organization of science and technology in China. The changes will affect all scientists, in the universities, research institutes and in industry, and will reward those institutes and individuals that contribute to China's scientific and technological development.

This development is no surprise. The reforms are themselves a reflection of the huge changes in social and economic life that have taken place under the pragmatic leadership of Deng Xiaoping. The reform began in China with the smashing of the Gang of Four and Deng's return to power in August 1977. By 1982 and the 12th Par-

ty Congress, he and his supporters were in complete control and the rhetoric and personality cult of Chairman Mao and his successor, "Wise Leader Hua", had been cast aside.

National goals now seem to have less to do with ideology than with making rapid material progress. The primary aim, set by the 12th National Congress, is to "quadruple the gross annual value of China's industrial and agricultural output by the end of this century and then proceed to raise, within the first 30-50 years of the next century, China's economy close to the level of the developed countries".

To achieve these goals, Deng has trans-

Individual responsibility taking over from egalitarianism?

THE "responsibility system", which has lately given Chinese peasants the right to sell food crops for what they can make in the free markets, also applies to the research enterprise, but in ways that seem to vary in a bewildering fashion from one institution to another. This is why the State Council's Leading Group on Science and Technology is even now drafting regulations to determine what should happen to the financial rewards of greater collaboration with industry. Wu Mingyu, vice-chairman of the State Science and Technology Commission, hopes that the rules will be ready by 1986.

At present, the practice is that a sizeable proportion (usually a half) of the money received under industrial contracts is passed on to the parent organization, the Chinese Academy of Sciences or the Commission on Education. It also seems that individuals can receive between 5 and 8 per cent of the income earned from industry by way of higher salaries, although these awards will certainly be shared by the whole of the research group concerned, perhaps the whole of a department and sometimes even by the whole staff of an institution.

Thus at the chemistry department of the University of Beijing, 30 per cent of outside income is handed over to the university and

rather more than 40 per cent goes on further development (which means research equipment and consumables). Personal awards amount to less than 10 per cent of the total, and are shared out among the whole of the department's staff *pro rata* with salary.

There is some chagrin at the high rate of tax on such payments. Annual supplements of less than three months' salary are paid gross, but if the amount of a person's extra payment should be 6 months' salary, the department is required to pay over three times as much to the state, thus sharply reducing the total available for awards.

At the Academy's Institute of Microbiology, the practice is different. Outside income is shared equally with the Academy, while 30 per cent of the receipts is used to cover research costs (so reducing the recurrent cost to the Academy). Between 5 and 8 per cent of the institute's share (roughly 500,000 yuan last year) can be used for making awards to individuals responsible for the research concerned, while the remainder is spent on project-related research.

Elsewhere, the novelty of the notion that research can spell money means that for the time being much more informal arrangements obtain. At the Institute of

Electronics at Guangzhou, which has just sold the software for a word processor program for manipulating Chinese characters to a Hong Kong company, for example, the local branch of the Academy seems not yet to have learned about the contract, and thus has not had a chance to say what should be done with the HK\$ 100,000 the institute now has sitting in the bank.

The rules now being drafted are likely to endow these arrangements, themselves quite novel, with a degree of formality. Wu Mingyu says there is a case for treating institutions differently according to the balance of their research, basic or applied. The obvious difficulty is that under the system to be introduced in 1986, when basic researchers will be expected to compete for support by means of formal applications to the National Science Foundation (see p. 210), the pattern of financial pressure will still leave even the best basic researchers with the knowledge that they would be financially better off working on a contract for an enterprise of some kind.

The obvious resolution of the difficulty would be to include salary supplements in grant awards. It will be interesting to see whether the leading group decides that some such solution, however inequalitarian, would be in China's best interests. As things are, it seems, anything is possible. □

formed Chinese life: the door has been opened to the West to encourage foreign investment; market forces, not central planners, are to determine the pattern of industrial production; peasants are allowed to farm their own land and to sell their surplus produce at free markets; and intellectuals and scientists have been given back their high status but not yet salaries to match. At the same time, many leftists who were promoted during the Cultural Revolution are being removed from office; the Peoples' Liberation Army is being severely reduced in size.

But the path to reform has not been uniformly smooth. In September, at a national conference of the Chinese Communist Party convened exceptionally between party congresses, Deng had to pay a high political price for his latest triumph, the replacement of more than sixty ageing members of the Central Committee by younger people, some of them conspicuously Deng protégés. For one thing, he found it necessary to emphasize in his own speech to the conference that reform should not be allowed to jeopardize Marxist principles, while the delegates also heard a sombre warning from another member of the Politbureau, the veteran Chen Yun, that the opening of China to the outside world, necessary though it may be on economic grounds, entails the risk of "corrosion by decadent capitalist ideology and conduct". Chen's speech was widely regarded in September as a demand that the reformers should be

more alert to the dangers of the course on which they had embarked.

In his capacity as first secretary of the Central Commission for Discipline Inspection (an organ mirrored at all levels of Chinese party society), Chen went on to demand that the Party should be "vigilant" in the fight against lawlessness, corruption and pornography. The "bourgeois decadent ideology of putting money above all is corrupting party conduct and social conduct in general", Chen said.

This is one sign that opposition to the process of reform, although now largely silenced, has not entirely vanished. Now, it is as if the process of reform itself is on trial. Its successes have been spectacular, especially in the countryside, but there have also been problems galore. The official statistics show that China is now entering its fifth consecutive year of growth at ten per cent a year or more, which is amply reflected both in the construction sites being worked in all the cities and by ordinary people's interest in consumer goods (refrigerators for the time being the most sought after). The other side of this coin is that imports are growing faster than exports, while many of the new projects on which the reformers have embarked have come to grief. These are now some of the reasons why the outline of the next five-year plan (1986-90), also agreed at September's national party conference, offers the more modest target of 7-8 per cent a year — twice the present rate of growth in Western Europe and still

enough to reach the target of quadrupled production by the end of the century.

On a more human scale, new opportunities for people to make money have led to some spectacular cases of corruption, even involving Party members. Some changes have had to be undone abruptly. Thus, in 1984, fourteen coastal cities were declared special economic zones, free ports of a kind in which foreign investment would be encouraged and in which, it was intended, joint enterprises between Chinese and overseas companies would flourish. By July this year, however, all but four of the economic zones had been closed, partly because they had not attracted suitable investment, partly because of the fear that corruption would get out of hand.

Inevitably, the rapid growth of the past five years has brought great inequalities. Perhaps for the first time in Chinese history, there are people in the cities who envy the peasants. The official response that "some must get rich first" does little to ease the tensions that have built up.

When the new pragmatic policies first surfaced in 1977, science and technology were seen as a force that could transform China. Soon science was to obtain a place of honour among the "Four Modernizations" (science, agriculture, industry and defence). And after years of being reviled among Mao's "stinking elements" (including landlords, capitalists and counter-revolutionaries), scientists and intellectuals suddenly became respectable again.

In 1978, Deng announced that "intellectuals are part of the working class", professional titles were restored and academic societies allowed to function again. In the euphoria of this "Springtime for Science" it was not surprising that over-optimistic plans were laid.

At the 1978 National Science Congress which confirmed the restoration of science, huge programmes were outlined in eight areas: agriculture, energy, materials science, computer science, lasers, space, high-energy physics and genetic engineering; and plans were included for a 50-GeV proton synchrotron and a fusion machine on the same scale as the Joint European Torus (JET). But within a few years, the limits of what could be achieved were realized, and attempts to aim straight for high technology were modified. On 13 March 1985, a new and thoroughly pragmatic series of reforms were announced by the "Decision of the Central Committee of the Communist Party of China" after some years of discussion with scientists.

The essence of the reform, entirely in line with general policy, is that "science and technology must be oriented to economic reconstruction". To that end, "the defect of relying on purely administrative means in science and technology management, with the state undertaking too much and exercising too rigid a control" must be remedied. The plan says that while research projects of national priority should remain under the control of the state, other activities conducted by the scientific and technological institutes are to be managed by means of "economic levers and market regulation". In other words, many scientific institutions and scientists are going to have to find customers to pay for their work. Many defects of the present system are frankly described in the decision document, and the ways they are to be remedied are made clear.

First, the system of the "great iron pot" is to be eliminated. Hitherto, the huge numbers of research institutes attached in one way or another to the government have yearly been doled out fixed sums of money for their services, largely calculated according to staff numbers. The key projects on which they are to work have also been determined by the government and allocated to institutes deemed suitable to carry them out. The result has been research institutes that are isolated not only from industry but also from one another, under little pressure to deliver results, and seriously overmanned.

Under the new regime, state handouts for research are gradually to be withdrawn. Instead, institutes are to "earn income and accumulate funds" by contracts, by transferring research achievements (to industry), operating joint venture and export (companies), and consultancy. The experiment has already begun. Some research institutes have seen their state income cut; soon the system will spread nationwide. The Party expects that some

institutes will not survive in the new competitive atmosphere and will have to close. Others may find it expedient to merge with related institutes or even with industrial organizations. All institutional innovations, it seems, will be encouraged.

Institutes carrying out basic research will come under a different kind of pressure. It is accepted by the Party that basic research must continue and that financial support cannot come from industry. But the costs of basic research (over and above the salary costs of those responsible) will no longer be provided automatically with the public subvention for an institute. Instead, there is to be a system of competitive grants open to all scientists in China, not only those who work in formal research institutes.

A start has been made on the development of such a system within the Chinese Academy of Sciences, which has set up a small science foundation to receive grant applications for researchers at its own institutes. These are subject to peer review, as elsewhere. Next year, according to the plans now laid, there will be a National Science Foundation for whose funds scientists from universities will be able to compete on an equal footing with those at research institutes operated by the production ministries and provincial governments as well as by the Academy.

As recently as early October this year, the constitution of the new foundation had not been finally settled, although there is every likelihood that the State Science and Technology Commission will have formal charge of it. But officials say that the chairman of the foundation will have to be an independent person. The scale on which the foundation will operate is similarly undecided, although it seems clear that an annual budget of less than several hundreds of millions of yuan a year will mean a cut in basic research support.

The new system will have radical consequences for the ways in which individual laboratories function. In many of them, people whose work can attract support from industry will be working alongside those who must look to the National Science Foundation for help with research costs. Even when grant applications succeed, basic researchers will not be entitled to the salary supplements that those working on applied research projects may in future enjoy when industrial innovations are taken up and exploited by industry.

Some fields will continue to be supported differently. Research institutes involved in medicine, public health, family planning, disaster prevention, meteorology and so on will continue to be supported by annual grants, but the plan is that the institutes will in future make proposals indicating which projects they wish to carry out. This is a departure from the present system, under which projects have been allocated from on high.

To get the institutes in shape for the new competitive world, they will be given con-

siderable decision-making powers of their own. During the Cultural Revolution, research institutes were run by "revolutionary committees" dominated by Party officials; ideology took precedence over scientific goals. Now, the power of scientific directors is being restored. Party officials are "to help with the effective application of the system under which directors assume overall responsibility and promote scientific and technical development". Indeed, a prerequisite for a Party committee secretary is now that he or she should "possess a certain level of scientific knowledge and be keen on scientific and technical undertakings and conscientious in carrying out the Party's policy towards intellectuals", a far cry from the days of the Cultural Revolution. Directors will thus now be firmly responsible for the scientific direction of their institutes and can, in principle, even sack inefficient workers.

There is also to be more freedom and greater rewards for the individual scientists. They will be allowed to do part-time consulting work and to keep the money they receive from it (minus any sums due for the use of state facilities). Large rewards are to be made to the individual scientist (rather than the institute) for major achievements (although there may well be pressure to share any such rewards out). And hitting at one of the most intractable problems of the present system, "rational job mobility" is to be introduced for "competent people must no longer be made to sit idle and waste their talent".

How far this reform will go remains to be seen; there are already semi-official newspapers in circulation offering job appointments; some show a huge range of perks for those willing to take their skills to organizations outside the big cities. But the present system of allocation to one's place of work upon graduation is still far from anything like an open job market.

What are the chances that the reforms can be put into practice? Accounts of how individual research institutes and universities are coping will be found in the pages that follow: the pace is faster in some than others, and some new kinds of profit-seeking technology corporations, unthinkable a few years ago, have sprung up. But China is a vast and bureaucratic country and changes at the lower levels of the hierarchy do not automatically follow central directives at any speed.

A story is already in circulation of a scientific director of a small and distant research institute who found, despite Party directives, that he could not wrench control over research from the local Party man. In the end he travelled to Beijing, appealed to Party authorities and returned with a high official who made it clear that the Party line was to be followed. No doubt change will be resisted at many levels by many different forces: at this early stage it is hard to guess what will emerge in the next year or two as the reforms take effect. □

Science policy

Low versus high technology

OVERALL responsibility for China's science and technology policy rests with the State Science and Technology Commission (SSTC). Like every organization associated with science, its fortunes have changed with the times: it was set up in 1958 during the Great Leap Forward, abolished at the start of the Cultural Revolution, and re-established in 1977 after the fall of the Gang of Four.

Since then the influence of the SSTC has gradually increased, at first under the leadership of Fang Yi, a very experienced politician and member of the Politburo. He did much to establish the SSTC's political respectability, but although he was college educated (unusual in the upper ranks of the Party), he had never been a research scientist. Now, the job of director general has gone to someone with a weighty scientific reputation: Song Jian, a 53-year-old cyberneticist, who trained in the Soviet Union, studied in the United States and took a leading role in the successful development of the space industry.

Most of the SSTC's departments are concerned with national policy, planning and legislation in science and technology. One of them, that for science and technology policy, effectively acts as a think tank for the Science and Technology Leading Group, the group of senior politicians in the State Council with special responsibility for making sure science policy matters receive top priority. And the SSTC also exercises ultimate control over the Chinese Academy of Sciences and ensures that government policy is carried out in a coordinated manner at other research facilities.

The SSTC does not itself maintain any experimental research facilities. Nor does it control directly the major part of the science budget. But it can combine the use of its own funds with the influence it has over allocations arrived at by the State Planning Commission (and given out by the Ministry of Finance) to provide effective control.

What this process has meant until now is the SSTC sitting down with the various science-related ministries and deciding on key projects of national importance that need special promotion — like those for

the development of the Yellow Loess Plateau, and fish aquaculture, for example — and then giving guidelines for how the funds should be allocated by the Ministry of Finance (on top of any routine allocations) as well as giving out the part of the budget it controls directly.

With the new reforms, much of this will change. The commission will still come up with key projects, but, as Lin Zixin, secretary-general of the SSTC put it, "instead of us selecting, we would like to let bids come from research organizations. Research, even on key projects would then be carried out under contract". Experiments are just beginning to be made with this new system, and if it turns out to be successful, then inefficient research organizations, unable to gain funds, will wither away. To sum up, "we used to choose from the top, the new plan is to be the reverse"; and at the same time, the new National Science Foundation will "cover a portion of basic research and applied research that can't produce practical economic results quickly".

This pragmatic approach comes along with a re-evaluation of what China can really achieve. Back in the heady days of 1978 many people were ready to believe that China could leap straight into an era of high-technology and catch up with the West.

Indeed, there are plenty of areas where China has done extraordinarily well: the manufacture of satellites, H-bombs and high-power electric generators are no mean technological achievements. The problem is that basic items such as bicycles, sewing machines and radio sets are of low-quality. Now there is an awareness that success in a few areas of high technology does not automatically feed into everyday products. Thus the SSTC has laid down five clear principles:

- to develop technology coordinately with society so that society can absorb its fruits and provide for its support;
- emphasize production technology;
- develop new technology in the industrial enterprises;
- in basic research stress China's unique problems;
- increase international cooperation.

These principles may sound unsurprising but they represent radical breaks with the past. Emphasis on research inside enterprises is a clear rejection of the Soviet model — independent state-controlled research institutes — which has been followed since 1949; instead it is now Japan, whose companies perform their own research, that provides inspiration. And emphasis on international cooperation is a complete break with the view held during the Cultural Revolution that "learning from abroad is a betrayal".

One problem still to be tackled is how to

get sufficient talent into industry — it will mean countering the prestige of the Chinese Academy of Sciences, for one thing. As Lin put it, "we have never paid enough attention to the needs of the enterprises, the best graduates were allocated to the institutes and government agencies. Although there is no difference in pay between factories and academia, people feel they are at a lower level if allocated to a small factory. This a complex social problem which is very difficult to change". But the carrot of financial incentives for those who work in industry is not yet under consideration.

It is clear that there is still debate over what technological level China should be trying for with some still believing that the "highest" technology is the best technology. Lin holds the view that it is selecting appropriate technology that is important. For example, in 1978 Chairman Hua



The State Science and Technology Commission's headquarters.

announced that 85 per cent of agriculture would be mechanized by 1988 — not a prediction that will be fulfilled. But Lin now argues that the use of draught animals and manual labour is actually much more rational; it should not be thought of as a sign of backwardness.

The planning of basic research is largely to be left to the Academy. But there will be some emphasis on solving problems specific to China. One example is research on rare earth elements in which China is particularly rich, and on which much basic research in refining remains to be done.

But whatever policy decisions are made there remains the awareness that shortage of technical skills, more than anything else, will determine the pace at which technological progress can take place. There are, according to the SSTC, 1.6 million enterprises in China but only 1.8 million "technical" staff who have received post middle-school training. In the electronics industry only 20 per cent of staff is trained, in the iron and steel industry 10 per cent, and in textiles and light industry 1 per cent. It is boosting these figures that will have to receive the highest priority of all.



Lin Zixin, secretary general of the State Science and Technology Commission.

Academy of Sciences

A pawn in revolutionary history

THE history of the Chinese Academy of Sciences is a mirror to the history of New China; for better or for worse, each change in policy has affected the Academy's fortunes. The organization was founded in November 1949, just one month after Mao Zedong proclaimed the founding of the People's Republic in Tiananmen Square. First priorities were to build an effective scientific research community; the Academy began with just twenty institutes and 300 research staff inherited from pre-revolutionary days. A major source of help was scientists who returned from overseas inspired by the desire to help China's development. A new science press was soon established and a major library; branches of the Academy were built up throughout China and academic relations with foreign countries — mainly in the Eastern bloc — were established.

In this early period China astonished the world by the moderation of its policies. Land was redistributed to the peasants and factories gradually nationalized, with compensation paid to the owners, giving China the highest growth rate in Asia.

In 1956 enthusiasm for science intensified when Mao Zedong called for science to lead the country forward and gave freedom to intellectual enquiry with the famous slogan "Let a hundred flowers bloom, let a hundred schools of thought contend". A 12-year science plan was also made with emphasis on natural resource exploration and on catching up with the West in key technologies — atomic energy, semiconductors and jet aircraft. All this helped to generate enthusiasm for science among the people.

But that enthusiasm was soon to get out of hand. In 1958 the Great Leap Forward began. Mass enthusiasm for science led to wild optimism about what it and China could achieve. The slogan was "Catch up with the USA, overtake Britain". As revolutionary fervour was intensified by mass campaigns, millions of peasants were reorganized into huge communes, massive irrigation projects begun, and dams built. The effects were felt in the laboratories; normal work ended, wild claims were made for all sorts of fantastic inventions and many people spent their time trying to put into effect the revolutionary sayings of the Chairman rather than doing research. In some laboratories the "backyard blastfurnaces" that Mao had claimed would allow rapid modernization were set up. And those who had spoken out during the "hundred flowers" period now found themselves under attack as rightists. Overall there were some gains for science in the enthusiasm for it which spread among the people, but the disruption prevented any real progress being made.

The dominance of the radicals ended as the Great Leap Forward ground to a halt in a morass of inefficiency compounded by some of the worst natural disasters China had experienced for a century.

In 1961 many tens of thousands of peasants starved to death in the countryside. And the year before, China had finally broken with the Soviet Union over the latter's "revisionist" policies and "friendship" with the United States, resulting in withdrawal of all technical advisors.

These disasters were quickly followed by a swing from the radical to more pragmatic policies. Mao Zedong lost his post as Head of State to Liu Shaoqi and Deng Xiaoping — now the most powerful man in China — became Secretary-General of the Party. These two, along with Premier Zhou Enlai did much to undo the worst aspects of the Great Leap Forward; some private plots of land and financial incentives were introduced in order to improve efficiency.

For the Academy it was a period when the role of scientists was made clearer. Guarantees were given that scientists could spend five out of six days in their laboratories (instead of at political classes): gone was the time spent on writing poems to praise Mao, practising in the militia, performing manual labour along with the peasants and all the other things that participation in mass movements had required. Also it had become clear after almost ten years of turmoil that there simply was not enough food for the Chinese people. Agricultural research now proceeded at top speed and real gains in food output were made. By 1964, science was flourishing as never before. In 1965 the Academy succeeded in launching a satellite, the world's first chemical synthesis of insulin was completed at the Shanghai Institute of Biochemistry and major advances were made in atomic energy research. But then the Great Proletarian Cultural Revolution began.

For some years leftists associated with Mao Zedong had been attempting to regain power through a Socialist Education Movement intended to mobilize the peasants to greater revolutionary ardour. Then in July 1966, after more than a year in which he had made no public appearances, Chairman Mao, then 73 years old, was seen in television pictures broadcast worldwide taking a "ten-mile" swim in the Yangtze river. The next month the Red Guards appeared in strength. Deng Xiaoping and Liu Shaoqi fell from power, the latter to be branded a "renegade, traitor and scab" and to die in disgrace. Deng spent two years in solitary confinement before being exiled to Jiangxi Province.

The ten years that were to follow were

Leading light in lasers

ONE of China's laser researchers remarked that there are more people studying lasers in China than in the rest of the world put together. Although said with a laugh it might even be true; one problem in China is that too many scientists cluster around a fashionable topic (a phenomenon dubbed the "swarming of bees") and then, because institutes are isolated from one another, proceed to duplicate low-level research being done elsewhere.

The Academy's Shanghai Institute of Optics and Fine Mechanics is not among those doing low-level research; it is at the top of the laser tree. It is not actually in Shanghai but in Jiading, more than an hour's drive out in the country where it was built in 1964 as the first part of a science city. The Cultural Revolution then came along however and the city was never completed.

The institute now has 600 researchers, 500 technicians and administrators and some worthy achievements. Among them are the development of inertial confinement fusion systems, a three million joule per pulse high-energy laser, and excimer lasers. Basic research at levels no different from that in the West is going on in laser



Professors Fang Honglie, Bi Wuji and Wang Kewu (left to right) stand in front of the target chamber for their six-beam, 10-watt a pulse, inertial confinement fusion research machine.

spectroscopy and atomic hyperfine structure. But even so the institute is under strong pressure to earn its own keep.

This year its funds were cut by 23 per cent and further annual cuts of 20 per cent are expected. The institute has reacted in two ways. First, industrial contracts are being sought and manufacturing facilities established. Fortunately the institute grows very good Nd laser rod glass as well as Yag, which has already been exported to Australia. Other commercial possibilities lie in laser writing of integrated circuit masks and medicine. Second, the institute is seeking "open" status: extra funds will be received from central government to allow scientists from all over China to come and run experiments, in a scheme administered by a national committee. And of course, institute scientists will be applying for funds from the new Science Foundation. □

Academy's computer centre really means business

THE "Technology Service Corporation of the Computing Centre of the Chinese Academy of Sciences" could do with a new title, as its general manager Yi Hui-en readily agrees. For far from being some sub-section of a research institute, the corporation is really a "high-tech venture business" of a kind unimaginable in the China of a few years ago.

The corporation began life in the summer of 1981 after the policy shift to a "commodity-based" economy was announced. Its aim was to train technicians who would then liaise with scientists in the Academy's research institutes and then help turn results into marketable products. Initial capital — just 50,000 yuan — came from the Academy. Senior middle-school graduates were provided to staff it, for at that stage an allocation of university graduates could not be obtained.

Despite these rather unpromising beginnings, the corporation has since generated 5 million yuan in profits and long ago paid off its loan. Now, not a single yuan is received from the state; everything including operating expenses and salaries is paid out of profits. And the profits ratio would make many a Western capitalist green with envy — last year profits of some 1.6 million yuan were achieved on a turnover of just

4.6 million yuan.

The corporation was one of the first of its kind in the field but there are now more than 200 similar organizations nationwide. All have the dual aim of "turning research results into productive forces" and "maintaining technological expertise, particularly through the training of technicians".

This particular corporation has made its money by catching onto the computer boom; others operate in fields as diverse as cell culture and new materials. Its biggest financial business is selling hardware, mostly small computers, but many of its 170 staff are designing software packages. It has set up a joint software venture with a Japanese company, is operating five factories in the Special Economic Zone bordering Hong Kong where a micro-computer is assembled for a US company and is also manufacturing automation control devices (programmable counting devices and the like). Now it is diversifying with a factory to produce vitamin oil by a method developed in Academy institutes in Hefei.

The corporation's staff claim they have complete freedom to launch new projects and choose new areas to explore. Ideas come from its own "think tank" containing scientists and engineers from various insti-

tutes. It is not restricted to working only with the Academy and links are formed with any research institute that might be able to contribute a marketable product. Diverse links are thus possible and it is hoped that more joint ventures will be launched with foreign countries. Now the corporation is a success, university-trained staff are being allocated to it for further training, and it may also be given the right to confer higher degrees.

Right now the only obstacle to further growth is lack of capital. But these are perhaps the golden days. The market is still firmly in the sellers' hands — such is the shortage of expertise that the corporation never needs to go out of its way to advertise its wares. But the prospect of growing competition for customers with other corporations is on the horizon.

Where then will be the boundary between communism and capitalism? No specific answer seems to exist. Perhaps the best that can be done is to quote Manager Qiao, a fictional character whose adventures were serialized in the *Workers' Daily* a few years ago. "Of course", Manager Qiao says "competition between factories in China will be totally different from the cut-throat struggle in capitalist countries. . . we can't scrap socialist cooperation". □

the worst period for science and technology. At the outbreak of the Cultural Revolution the Academy had 106 institutes, by 1973 the number had fallen to 53. Some were simply closed altogether, others were taken over by regional authorities and others packed up and moved far into remote regions of China. Research staff had stood at 60,000 in 1965; by 1973 only one-third were left. Some 30,000 scientists were sent for re-education in "cadre schools" in the countryside. Those who were simply given physical labour in the country were lucky; in some institutes more than half the staff died as attacks on any kind of intellectuals intensified. In one institute a political appointee claimed that "knowledge of a foreign language" was sufficient evidence of being "a foreign spy".

But the terrible sufferings undergone by scientists and intellectuals during the Cultural Revolution are already well known. Mention of these days is now almost always greeted with laughter — not because there is anything funny to recall but because a laugh is a standard Chinese response to a memory that is too painful or too embarrassing to talk about.

The Cultural Revolution proper lasted only until 1968, for by then the activities of the Red Guard had grown so uncontrollable that industrial production was being disrupted. The People's Liberation Army, headed by Lin Biao, restored order and sent millions of young people to work in the countryside and to establish communes in remote border regions. But the

persecution of intellectuals and domination by the left was not to end so quickly. In 1971 Lin Biao died in an "aeroplane crash" after a failed attempt to assassinate Mao Zedong. Only gradually, under the influence of Prime Minister Zhou Enlai, were things restored to something like normal. In 1973 Deng Xiaoping was once again given power.

His return was to be for a few short years in which there was not time to undo the harm of the Cultural Revolution. At the same time as his reappointment the "Gang of Four", headed by Mao's wife Jiang Qing, had launched a leftist campaign. Then in January Zhou Enlai died, removing the last remaining moderating influence upon the radicals. A few months later Deng Xiaoping was again dismissed from power. It was then that fate intervened — in September 1976 Mao Zedong died. Within weeks the Gang of Four were under arrest and the pragmatists were back in power. By August 1977 Deng Xiaoping was in high office for the third time.

Although the worst excesses of the "leftists" were over by 1973, very little progress was made in rebuilding the scientific framework between 1973 and 1977 while the leftists and the Gang of Four had influence. But after the "smashing of the Gang of Four" (a standard phrase in China, and one always enunciated with particular relish) progress was rapid.

In 1977 the "Four Modernizations" were announced as the key objectives for the nation: modernization of agriculture,

industry, defence and science and technology. A national science congress was convened in 1978 and a highly ambitious eight-year plan approved which would once more make science and technology a major force in the nation. Some of the more ambitious projects have since been abandoned or scaled down, as described elsewhere.

By 1985 the Chinese Academy of Sciences was in better shape than ever before, with 117 institutes and 80,000 staff. Almost half are technicians and scientists and the remainder administrators, support staff and Party workers. A huge support staff is necessary partly because the Academy has to manufacture much of its own equipment at its eight factories — it even produces its own gases for use in research laboratories as there is no national supply facility.

Institutes dispersed during the Cultural Revolution have in the main returned to their old homes; an exception is the Academy's university, the University of Science and Technology of China, which was moved to the small city of Hefei in Anhui Province. A science city has grown up around the university, including a number of new research institutes, and there is now no longer any possibility of moving it back to Beijing.

Like other institutions, the Chinese Academy of Sciences is now busy implementing the new reforms. Several unique moves have been taken to overcome the difficulties spelt out in the reform document. To answer the criticism that

institutes are too isolated, some, or some subsections of them, are to become "national" laboratories open to other research workers throughout China. The idea is that a part of the staff will not be fixed but will be appointed from other institutes on one- or two-year contracts. A national committee with representatives from many institutes will appoint the director of the new "open" laboratories. Seventeen institutes have already been chosen for the scheme: they include the Theoretical Physics Institute and the Institute of Mathematics. Many other institutes are also hoping to gain "open" sta-

tus, in part because funds will be provided by the central government which could help offset income lost as the reforms begin to take effect.

Another move which the Academy has been taking over the past few years is to establish new high-technology corporations which can market ideas developed in the Academy's institutes; one, described in the pages that follow, is principally concerned with selling hardware and developing software; others have spun off from botany into cell-culture technology, and from chemistry into the manufacture of new materials. □

rationality. At present the SCE has 36 universities under its direct control, many of them "key" universities which attract the most funds and the best students. Other universities belong to other ministries; some of the best of these (for instance the University of Iron and Steel Technology of the Ministry of Metallurgical Industries) are managed jointly by the SCE and the relevant ministry, but most of them are exclusively managed by their own ministry. Graduates from these latter universities — or around 80 per cent of them — are sent to the parent ministry's offices, colleges and factories to work. Only the remainder may go elsewhere.

No-one feels that this divisive system is really for the best; but change is sought that goes beyond just making all universities answerable to the SCE. Instead, the Party wants to see a loosening of links between all universities and their ministries, including even the SCE; just as there should also be a loosening of links between factories and ministries. Indeed, going higher up the hierarchy, the Party would like to see many ministries done away with and turned into state corporations. A few years ago, that was what happened to the Ministry of Shipbuilding; it became the Shipbuilding Corporation to "overcome bureaucracy and to practise free competition".

How far reform can go in the case of the universities is hard to predict. Many academics are sceptical, one commenting that "it cannot be changed", but then adding "but maybe Li has the authority to do it".

Li will also have his hands full in simply trying to get the whole educational system close to world standards. In 1981, there were around 1.279 million students at China's universities, 1.061 million of them at four-year universities and the rest pursuing two-year courses. Now the total is close to two million. In the Philippines, however, with a population of just 48 million, there are 1.8 million university students. Things are certainly getting better and this year, half a million university places will be available for three million applicants, but there is still an enormous distance to go.

Further down the system problems are grave. Before university comes three years senior middle school, three years of junior middle school and six years of primary school. The goal being fought for at the moment is universal primary school education. Only wealthy Jiangsu Province has so far been successful. Nationwide, 94 per cent of children enter primary school, but only two-thirds complete the first six years of schooling, with drop-out rates very high in rural areas. The hope is that primary school attendance will be made compulsory by 1990. One problem is that education, even at the primary level, is not free and many peasants want their children at home working on the land. □

Education commission and universities

Catching up the priority

EVER since the founding of the People's Republic, it has been the Chinese Academy of Sciences, not the universities, that has dominated the nation's basic scientific research. In terms of facilities, time available for research and funds the universities have been poor cousins to the Academy. But the new reforms and the rebirth of the Ministry of Education as the more powerful State Education Commission (SEC), are likely to give the universities a more equal chance.

Commission status was gained in the middle of June this year and in July, when a visit to the commission was made, the paint on the new signboard outside the building was scarcely dry. New policy decisions had not yet been made in any detail. One thing that was clear, though, was that the new head of the commission, Li Peng, is a powerful figure.

Li is one of the nation's four vice-presidents and holds a number of other ministerial appointments, including responsibility for energy. In September he was one of the "new generation" to be appointed as a member of the 24-man Politburo. Many believe he is on the way to the very top and at 57 he is still young in Chinese terms. Li is likely to be able to get things done and to be able to overcome bureaucratic resistance to some much needed reforms.

Why university researchers feel reform is necessary can be seen by a glance at the

table shown below.

Although the Academy takes a share of the state budget for natural sciences research many times larger than that taken by the SEC universities or universities attached to other ministries, it does not win the majority of State Science and Invention Prizes. Nor does it have a majority of highly qualified staff. Of course, neither of these measures can be said to unequivocally measure scientific ability, but the disparity in resources allocated to the universities and the Academy has been a source of friction for more than 30 years.

From next year, university scientists should get more of an equal chance to prove their worth. A National Science Foundation will be established in 1986 (although who will run it has yet to be settled by the State Council) and university researchers will then compete for grants with researchers from the Academy and elsewhere. They will also be able to bid for contracts for large research projects that would formally have been allocated by the government. The new foundation will probably have around 100 million yuan at its disposal for the first year, three times the funds of the present Academy foundation.

Bringing about changes of this kind will be just one task for the SCE, and a fairly minor one at that. More important is that the whole of higher education be managed

The natural sciences in China 1984

	SEC universities	Chinese Academy of Sciences	Other universities
Full and associate professors	10,765	2,956	26,299
Doctoral students	316	140	233
MSc. students	13,207	2,885	16,815
Science and Invention Prizes	163	152	112
Central government budget (million yuan)	20 (160)*	460	30

*Including funds from industry for research in natural sciences. The above figures do not include salaries or major capital expenditures.

Life as a student

THE exact conditions that students face at university vary from place to place: some have introduced Western features in their curriculum and assessment methods, others adhere to a Soviet model and a minority have not yet recovered from the effects of the Cultural Revolution. But political classes, which once dominated university life, are down to 72 hours a year in the key universities.

One constant factor seems to be that all students live on campus, generally six to eight to a dormitory with bunk beds and one long table and bench being the norm. At graduate level, more space is provided and students live two to three to a room.

At Qinghua University a US-style grade system has been adopted. Each term the available courses, teachers and hours of lectures are announced along with the number of credits that can be gained towards the total needed for a degree. The course is five years, rather than the usual four, and a final-year project is seen as of key importance. That project might be something like taking an old theatre and making a plan for its restoration. Once all that is completed, the student faces an oral exam: 20 minutes to describe the project and then 40 minutes to defend it under questioning from the staff.

Entry into graduate school is not, however, gained by obtaining a good degree. A quite separate nationwide examination is taken in the winter of the final year, with the results announced in April. The successful student does not have to stay on at the same university for graduate work but 70 per cent do so.

Those who do not go on to further studies will be assigned jobs on graduation. In the old days (just a few years ago), students had no choice over where they would be sent to work. But nowadays lists of jobs are usually circulated, with the best students given first choice. The lists are prepared by the State Planning Commission after meetings at provincial level to collect requests for the highly trained products of the key universities. Once a student has been appointed to a work place, he or she may have little chance of changing it. The system seems widely accepted by students (as is the extreme difficulty of obtaining permission to live in Beijing or any of the big cities) as being necessary for the "good of China"; but that does not stop people using every trick in the book to make sure it is someone else who is sent to a small factory, far from the capital.

Yet another examination awaits those who wish to go on from graduate school to a doctoral course. But those who do go through and complete the doctoral course are virtually assured of a lectureship: as doctoral courses began again only a few years ago, there are still only a few hundred with such qualifications in the whole country. □

University entrance

Cramming to join the elite

THIS year around half a million university places will be available: more than ever before. But with three million students likely to be taking the entrance examination, that still means tough competition, especially for entrance into the elite key universities.

For universities at the very top like Qinghua, Fudan and Beijing, successful applicants will need to achieve a score of somewhere around 455 out of 600 (76 per cent) in the six-paper, 100 marks a paper entrance examination. Papers can be taken in physics, chemistry, biology, history, geography, Chinese, English, mathematics (with separate papers for science and arts majors) — and everyone takes politics.

Other key universities might be content with some twenty marks less. That the dividing line is so thin shows how fierce the competition is at the top. Marks count for everything as it is not possible to conduct interviews.

There is plenty of competition further down the education system too. Differences in success rates between different middle schools are huge: some "key" and university-attached senior middle schools send almost all of their students to university while another senior middle school just down the road may be able to manage only 1 or 2 per cent.

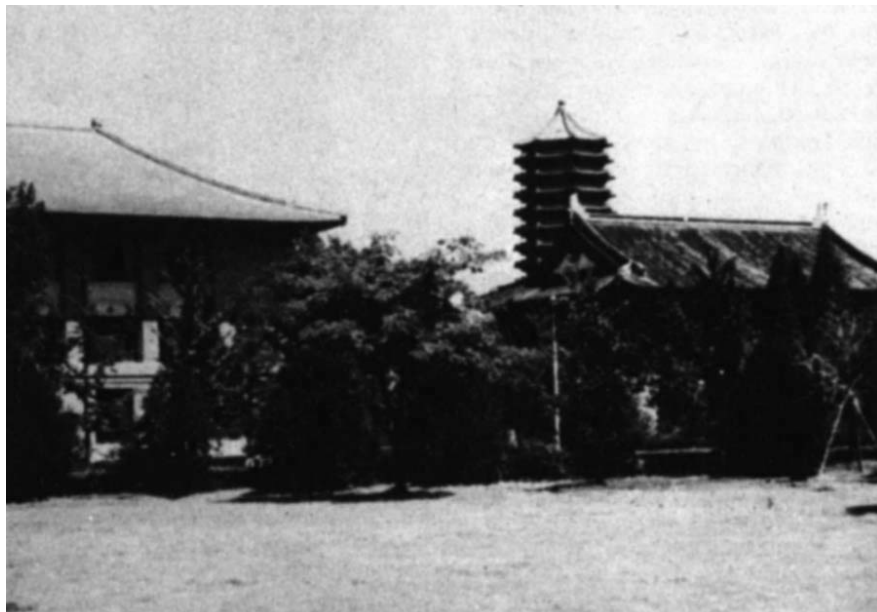
As one father with a daughter studying for university put it "it would be hopeless to try to enter university from a non-key school". Getting into a key school is again largely a matter of passing exams, although "influence helps"; competition at 12–13 years is tough, with parents busy helping with homework until ten every

evening. There are even "key" primary schools with preferred entry to key middle schools (although steps are being taken to do away with them). All this hectic studying will be familiar to the Japanese, as will another new development, the appearance of after-hours cramming schools. More than a few schoolteachers now run special private classes for those anxious to succeed.

The present system is a far cry from the Gang of Four days when the "soldier-worker-peasant" students were sent to university on the recommendation of their workmates; in those days there were no examinations (and nor were there degrees or graduate students).

There is still a small percentage of students who do not have to go through the entrance examination. At Qinghua, for example, around five per cent are instead taken in on the direct recommendation of their schools. Recommendations are taken only from a few privileged "key" schools that have built up close links with the university and have a tradition of excellence. Students will need to have the school president certify that their ability, character and health are truly exceptional before they have a chance.

Under consideration is the highly controversial suggestion that entrance requirements should be slightly eased for those that are willing to pay university fees. Indeed, it is even possible that most students will be asked to pay their way through university. It is for the best, in the long run, some say. The logic — pragmatic enough — is that for every student who pays, an extra student can be taken on and total enrolment increased. □



One of the best of China's educational institutions — Beijing University. Situated in the northwest of the city, most of the university's buildings are in the traditional Chinese style. The pagoda in the background is actually a water tower supplying the campus.

Intellectuals

Jam tomorrow — perhaps

YOUNG lecturers now entering the universities face considerable frustration and, like all intellectuals, have to cope on salaries well below those of industrial workers.

All university promotion was frozen several years ago while the government tried to sort out a new personnel policy. On top of that, retirements are not taking place as planned. In theory, staff should retire at 60 (if male) or at 55 (if female). Associate professors may continue until 65, and professors to 70, but they are not supposed to have administrative responsibility. However, these rules are hardly ever enforced. The majority of full professors are in their seventies or eighties and associate professors in their fifties or sixties. Perhaps the example recently set at the Party Congress, where the older generation retired from high office *en masse*, will have an effect!

Many argue that the older scientists have to stay on because the generation that should replace them missed their education as a result of the Cultural Revolution. But there seems to be no shortage of younger talent: there are many in their forties waiting for promotion and some have talented postdoctoral researchers in "cold storage" on scholarships overseas.

Whatever happens, a young lecturer is

certainly not going to live in luxury. Wages are only around 90 yuan a month. To put that in perspective, the national average salary is now 95 yuan a month, so a university lecturer earns much less than a factory worker. Top professors can earn 250 yuan a month, and university presidents as much as 330 yuan a month. But recently, much to the envy of city dwellers, peasants near big cities who have been selling their wares in city free markets have been reaching incomes of 550 yuan a month!

Indeed, the new rich can be spotted in the Shanghai suburbs, driving into town in the morning in privately owned trucks (which bear distinctive red number plates) loaded with vegetables and heading in the opposite direction later in the day loaded with cement. The neat little two-storey concrete houses they build now dot the countryside surrounding Shanghai.

Intellectuals will have to wait some while for similar luxuries. Although accommodation is provided at very low rates, there seems little sign of any pay rises on the horizon. No concessions were given to intellectuals in the current five-year plan and the word is that they will have to wait for their reward until the next. □

Biotechnology

Planning for a model institute

CHINA's first biotechnology centre is just a scale model at present, but within two years everything from research laboratories (including four P3 labs) and animal rooms to a library and accommodation for visiting scientists should be complete. Some 57 million yuan have been set aside for construction and US\$5 million for buying modern instruments and reagents from abroad. Shanghai, where the institute is to be, has thus stolen a march on Beijing where plans for a biotechnology research institute are still at the discussion stage.

Shanghai has capitalized on its rich biological research community in going ahead with the centre: most of the 130 young research staff will come from the large Academy institutes of biochemistry, cell biology, physiology, medical research and the like. But capital to establish the institute comes from the central funds of the State Planning Commission.

Much of the centre's research will be in "traditional" fermentation according to its vice-director Sun Yu-kun, who came from the Institute of Biochemistry. A conservative approach is being taken because capital is scarce and the institute must make money soon; genetic engineering has a long way to go before it turns a profit and can thus occupy only a small part of the centre's activities. Nor is there significant experience in "downstream" processing in China; first attempts are likely to be the production of small peptide hormones in yeasts. Growth hormone, which can stimulate milk yields, is a likely candidate as its chemical synthesis has already been achieved in China.

A more conventional project being given thought is to use immobilized enzymes to turn the galactose in milk into glucose and thus increase its nutritional value. Others are the production of vitamin C through fermentation, a process developed in Beijing's Institute of Microbiology; and the production of penicillin acylase — used in the manufacture of semisynthetic penicillins — by a method developed at the Shanghai Institute of Materia Medica. Wherever possible it seems that techniques already mastered in China will be built upon. But these are early days and it is not yet clear exactly what line will be pursued.

Links with foreign companies are also likely and the centre is close to a contract with a European company to produce both monoclonal and polyclonal antibodies as diagnostic kits; no such commercial kits are yet produced in China. Foreign staff are also to be invited to the institute, along with guest researchers from other parts of China. But Professor Sun seems a little pessimistic about further

On your bikes to Qinghua

REMEMBERING where you parked your bicycle can be something of a problem on Qinghua University's vast campus; there are ten thousand students and rows of identical-looking bicycles parked everywhere. Qinghua is the top "key" university for engineering (with different engineering departments covering every speciality) and took the Soviet technical university as its model. Its main building, seen here, is in the Soviet monumental style but elsewhere there are reminders of the university's origins. Traditional one-storey buildings with upswept roof-corners, vermilion pillars and classical rock gardens remain on one part of the campus, while a lecture hall with a domed roof and a Greek portico recalls the pre-revolutionary days when the university was a preparatory school for those going to study in the United States.

Also a surprise is that there are several factories on campus — the university even runs its own 2,000 kW power station. The factories are there for three reasons: to provide practical engineering training for students, to carry out developmental work, and to produce experimental equipment and manufacture goods for the market. Qinghua is successfully making a small computer, three types of micro-computer, machine tools, electronic components and precision tools. Research is often supported through contracts with in-

dustry, around 90 per cent of the university's research funds are gained in this way.

One of the university's top priorities is to improve the standard of teaching. Too many staff are poorly trained (blamed on the turmoil caused by the Cultural Revolution) and classes crowded, with lectures



Qinghua's parking problem outside the main university building.

attended by 100–200 students and seminars by 25–30 students. But noticeboards advertising lectures by visiting foreigners show there is considerable fresh input. □

prospects for joint ventures; foreign companies often just want to sell their product or want too much money for their patents — even though they are interested enough in the China market.

In the longer term, there are hopes for a hepatitis B vaccine. With 10 per cent of the population carriers the need is obvious. But there is much work to be done before there is any hope of mass immunization for costs are still far too high.

Shanghai Institute of Biochemistry

The molecular biology revolution

SHANGHAI'S Institute of Biochemistry gained world-wide renown in 1965 when it brought off the complete chemical synthesis of insulin — a technical *tour de force* requiring a seven-year team effort. After insulin came the chemical syntheses of vasopressin, oxytocin, angiotensin and luteinizing hormone releasing hormone (LRH); and then in 1981 another technical triumph with the total synthesis of yeast alanine transfer RNA.

But now the institute's interests are turning more towards molecular biology. Indeed, this would have happened a lot earlier if it had not been for the Cultural Revolution. During that period the institute's fame enabled it to continue in operation; but only to perform the same kind of research. In the world outside molecular biology was blossoming but there was no chance of absorbing "foreign" learning.

Professor Zhang You-shang, who now heads the institute, and his colleagues are well aware that the real value of chemical synthesis is in structure-function studies; during the Cultural Revolution they had to treat synthesis as an object in its own right.

Rapid progress is now being made in

A nationwide plan is also being worked out for biotechnology by the State Science and Technology Commission and considerable stress is expected to fall on applications in agriculture and aquaculture. But Beijing's plans to set up several more biotechnology centres may fall flat for want of trained staff. Professor Sun, at least, believes it better to steadily expand one institute than waste resources on competing institutes. □

molecular biology. A sub-type of the hepatitis B virus (not the one found in the West) has been cloned and sequenced: the surface antigen was subsequently cloned in both *Escherichia coli* and yeast, but in neither case was expression sufficient for use in vaccine manufacture; vaccinia virus is looking more hopeful with clinical trials perhaps a year or two away. Numerous other projects are under way.

Problems of a different kind also much occupy the mind of the director who is in the midst of reconstructing the institute in line with the new reforms. What should emerge is a slimmed-down and perhaps stronger research institute with a brand-new molecular biology laboratory.

First, overstaffing has had to be tackled. During the Cultural Revolution, the institute grew larger and larger, with even unqualified senior middle school graduates sent to do research. There was no incentive to keep staff numbers down for funds were allocated simply according to the number of people employed.

Now that there is a financial squeeze on the way, the director is having to exercise his new powers of control over institute affairs. The institute has been divided into 39 research groups, with their leaders

appointed by the director. This did away with one level of hierarchy — the division — which previously isolated research groups. And the number of researchers in each group is now strictly limited. Researchers are appointed by group leaders on three-year contracts. But what is to happen to the excess researchers? If the new reform documents were interpreted literally, they could be sacked. But that is actually out of the question; a little persuasion has to be used to make them move elsewhere.

In essence, what is happening here and in other institutes is that those who are not



The legacy of the Cultural Revolution adds to Professor Zhang You-shang's staffing problems.

chosen to join in a research team receive neither space, equipment nor research funds although they will still be entitled to their salaries. Such "people to be appointed" as they are called, are given every help to find work elsewhere. But they will not be sacked.

Special difficulties come with some of those sent to the institute during the Cultural Revolution on the recommendation of peasants, workers and soldiers but who do not have formal qualifications. Some have proved successful but as Professor Zhang put it "some are not so suit-

THE meeting hall of the Chinese Academy of Medical Sciences is this magnificent old building just a stone's throw from the "forbidden city". The Academy's headquarters are just across the road, alongside the Capital Medical College which provided the base from which the Academy sprang. The hospital itself was founded back in 1921 and has been through a number of name changes according to the political climate — during the Cultural Revolution it had a brief spell as the "Anti-US Imperialism Medical University". The Academy now runs 19 research institutes, most of them in Beijing with two of the biggest, Clinical Medicine and Basic Medicine, on the central site. Nine thousand staff are employed with 400 at the professor and associate professor level. Strongest research areas are in cancer, particularly epidemiology and prevention, and cardiovascular diseases. But major weaknesses remain in basic medical research and biomedical engineering. □



able, but it is not so easy to transfer them now, they are not so young. If they transfer elsewhere they will have to start again at the beginning". Such workers are likely to stay on for a while doing various temporary jobs. What kind of personal strains these readjustments are causing — and they come on top of personal animosities that must have developed during the Cultural Revolution — Professor Zhang did not say, but one sensed that the burden of his task is not light.

There is good news for the institute too, though, in that it is to have a new Laboratory of Molecular Biology, funded by the government and the Chinese Academy of

Sciences, with the aim of providing facilities every bit as good as in Western countries. Indeed the laboratory's budget is to be as large as that of the parent institute itself. If anything, it is to be modelled on Cambridge University's laboratory of the same name, the permanent staff is to be small and visiting scientists, including those from abroad, are to be encouraged to come on contracts of 1–3 years. Three floors of the institute will be given up to the new laboratory; some of the changes needed to set it up are very simple: just installing air conditioning will make it possible to do research all year instead of having to stop in summer. □

Cancer epidemiology

Mapping the mortality risks

EVEN if China is a developing country, the major causes of death follow patterns very similar to those of the developed countries. So cancer is a major concern and, with hepatitis and birth control, is one of the three priority research areas earmarked by the Ministry of Health. Epidemiological research is given particular attention, partly because epidemiology leads the way towards prevention and partly because of the publication in 1979 of the *Atlas of Cancer Mortality in the People's Republic of China* (China Map Press), which revealed striking patterns of incidence of the different cancers across the country.

The atlas is an organizational tour de force. The survey on which it is based, carried out between 1973–75, covered 96.7 per cent of the population (approximately 800 million people). Ironically, the dispersal of many professional people to the countryside by the Cultural Revolution probably helped to make the compilation of the atlas possible and so may represent one positive outcome of this unhappy time.

Although the total cancer mortality (approximately 25 per cent of deaths) is similar to that in developed countries, the atlas shows that the overall frequency of particular cancers differs markedly from those common in the West. Stomach cancer is the most common form for both men and women in China. For men, oesophagus, liver and lung cancer follow in that order of frequency, while for women the order is cervix, oesophagus and liver. In Britain, for example, lung cancer is the most common among males followed by colorectal, stomach and prostate cancer. For women, breast cancer is followed by lung and colorectal cancer.

That environmental factors may be important is suggested by the marked variation of frequency in different parts of the country. Overall rates of death from cancer are highest in the south-east coastal provinces, in Shanxi in central China and in parts of the border regions with Mongolia. Nasopharyngeal cancer shows a strik-

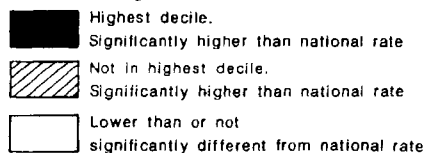
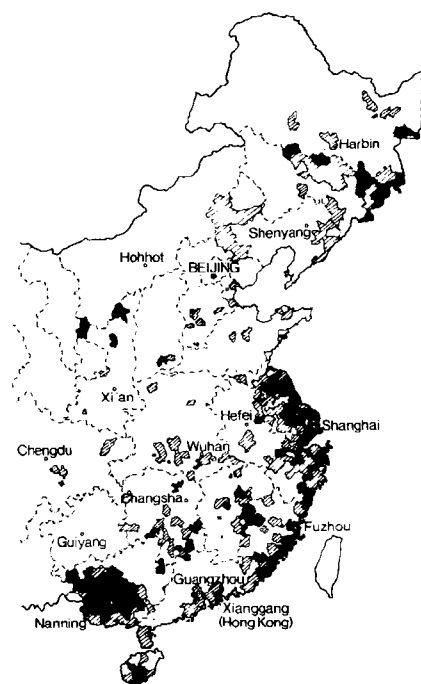
ing focus in Guangdong and Guangxi provinces in the south where it is almost as common as stomach cancer, whereas in Xinjiang Uygur in the far northwest, it is rare. Cervical cancer shows very high rates in Shanxi and inner Mongolia. Liver cancer (see map) is highest in Guangxi and along the south-east coast — interestingly, across the water in Japan these cancers are also a major concern. Other notable features include cancer of the penis, admittedly rare, which occurs to a large extent in the areas of highest incidence of cervical cancer.

This huge database, together with accumulating knowledge of known or suspected cofactors — hepatitis B virus (HBV) in liver cancer, Epstein-Barr virus in nasopharyngeal cancer and smoking in lung cancer — have fuelled a search for other factors which may be important.

Sun Tsung-tang at the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, is investigating the possible role of aflatoxins, the fungally-derived contamination of cereal foods. In Qidong county, Jiangsu, high levels of aflatoxin contamination of foodstuffs have been found. Liver cancer rates are also six times those in Beijing, but the frequency of HBV carriers is only 2.5 times as much. Sun plans a four-year study which aims to reduce dietary exposure by screening foodstuffs with monoclonal antibodies against aflatoxins and by chromatographic techniques he has developed.

The role of aflatoxins is also being investigated by the Shanghai Cancer Institute on Chongming island, in the Changjiang (Yangtze) delta, again a region with high rates of liver cancer. In a longitudinal study of 40,000 people, details of diet are being combined with an extensive investigation of aflatoxin contamination.

Shanghai has had a cancer registry since 1963. The city has very high rates of stomach, liver and oesophageal cancer in both sexes, but, most notably, very high rates of female lung cancer. About 50 per cent of Shanghai males smoke cigarettes and the attributable risk of smoking for



Age-adjusted male liver cancer mortality for 1973–75 in relation to the national rate.

lung cancer is 80.5 per cent. On the other hand, only six per cent of Shanghai women smoke, and the corresponding association is as low as 19.3 per cent. With poor ventilation and traditional practice, there is a possibility that cooking oil fumes may account for some of the high female incidence. Gao Yutang of the Shanghai Cancer Institute is carrying out laboratory and epidemiological studies; a causal study of 220,000 adults was begun in 1983 in collaboration with the US National Cancer Institute.

Dietary factors have also been implicated in stomach cancer. Zhang Rufu of the Beijing Institute for Cancer Research has analysed gastric juice from a total of more than 40,000 people in high-risk regions such as Gansu province in the north-west and Fujian in the south-east as well as in low risk regions elsewhere. High risk is associated with high nitrite levels and the fungus *Aspergillus versicolor* in the stomach. Atrophic gastritis is also more prevalent in these areas, possibly as a result of poor diet which, at the end of winter, can be dependent to a large degree in some areas on contaminated pickled vegetables.

It is too early to have a clear picture of the role of dietary and other environmental factors in the development of cancer, but given the heterogeneity of diet and surroundings in China and the heterogeneity of cancer incidence shown by the atlas, much may yet be revealed.

Nigel Williams

Polytechnic education

University as industrial partner

THE Beijing Polytechnic University (BPU) thinks of itself as China's version of the Massachusetts Institute of Technology (MIT), perhaps not now but sometime. It has a number of competitors, most obviously the Chinese Academy's own University of Science and Technology, transplanted south from Beijing to Hefei during the Cultural Revolution. In the long run, BPU's advantage may be its location in Beijing, which is where most people seem to want to live.

The university lives on a sparkling new campus in an industrial suburb of the capital. As in much of the rest of Beijing, buildings wreathed in scaffolding are a sign of things to come. The university's president, Professor Fan Gongxiu, is able to boast of a close relationship with the municipality of Beijing that should keep his institution in the forefront of the city's expansion for some time to come. And as at MIT, there are more tangible achievements to brag about — a state prize for the development of a laser printer (now being manufactured at Barbin, in the north-east) for example, and the second prize in the national Go competition. (Go is the



Dong Zhichang (right) and Zhang Zhi of BPU's Chemistry and Environment Engineering Department won a Beijing Science and Technology Achievements award for developing a crystal glaze.

board game sometimes counted as the Chinese equivalent of chess.)

By some other standards, the university is small. Student enrolment is 3,800 (of whom 300 are graduate students), but is expected to reach 4,500 by 1990. Usually, BPU sets out to recruit students who have already started work in industry, some 500 of them this year. As at other Chinese universities, the ratio of students to staff is luxurious (there are 1,250 members of the teaching staff, 750 of whom are professors or lecturers, and a roughly equal number engaged on other duties); the explanation is that a student's enrolment entails total responsibility; students must not be merely taught but housed, supervised and even instructed in what is called "political science".

Stimulated by an experiment in educational reform launched in 1983 by the municipality of Beijing, the university has paid special attention to "moral education", which takes the practical form of

engaging students on social work among old people and the like.

The most striking sign of what the university does for industry is the source of its funds; this year, 8 million yuan will come from the central government but 6 million yuan from industry. On top of that, the university has this year brought in 4 million yuan for research, chiefly as research contracts with industrial enterprises.

The demand for the university's services as an industrial partner has meant that 20 substantial projects have been turned away this year. Selfconsciously, the university reckons to be able to choose the projects with the "best economic value", but a collaborative research project will not be undertaken unless there is a suitably qualified member of the faculty who is, moreover, willing to take on the responsibility.

So what impression of the quality of industrial research in China has this well-placed university formed? Professor Fan acknowledges that many (but not all) industrial enterprises are much in need of skilled technical people, but he does not agree that industry needs always to be capable of carrying out its own research and development. That may be the pattern in the West, but in China it may make sense, and economize in scarce resources, that institutions like BPU should take on central research and development for a group of industries.

Often, it seems, the intimacy of the relationship threatens to turn the university into an industry in its own right. In 1981, Fan says, the industrial design department at the university embarked on a study of single-board microprocessors, from which sprang no fewer than a score of novel designs. So now the university has a factory with 100 workers which, last year, earned 20 million yuan from the sale of its products. Among other things, Fan says, the factory is a model for other factories elsewhere in China.

The curriculum at BPU is rigorous, perhaps even more so than at MIT. The standard course lasts for five years, beginning with basic science instruction. There is a language laboratory to ensure that everybody has a chance to learn some language other than Chinese, and facilities for art instruction and practice. Project work plays an important part in the curriculum in the later years of the course, and BPU reckons to do exceptionally well in the national competitions regularly organized in China for those with engineering innovations to show off.

For a university as new as this (BPU came into being only in 1960, directly under the wing of the municipality, and most of its work was suspended during the Cultural Revolution), international relationships are prominent and strong, espe-

Fighting restrictions



LIANG Zhiquan (left) and Shen Yufei in the Department of Molecular Biology and Biochemistry at the Institute of Basic Medical Sciences (Chinese Academy of Medical Sciences), Beijing. Founded in 1919 as part of the Beijing Union Medical College, it now has a scientific staff of around 370 and the largest department is that of molecular biology.

The emphasis is basic work on well-defined Chinese medical problems. As in Shanghai (see page 217), a hepatitis B virus subtype has been cloned and sequenced — an essential step towards the production of a recombinant vaccine. The use of gene probes to study and detect prenatally α - and β -thalassaemias, which are quite common in Guanxi, Guangdong and Sichuan, is being developed by Wu Guanyu in collaboration with other institutes in the affected regions. Successful prenatal diagnosis of α -thalassaemia has been carried out.

A new and important role for the institute is the development of restriction enzyme production and Qiang Boqin is keen to hasten the time when China can be self-sufficient in these reagents.

Progress at the institute has somewhat overwhelmed the old buildings, but hopes are high that brighter more spacious accommodation will be available in a new building within four years.

Nigel Williams

cially with institutions such as the State University of New York at Buffalo, Northeastern University and the like. One objective is to let potential members of the Beijing faculty complete their studies in a stimulating environment, another to add to the university's competence in industrial research.

Among the young people of Beijing, BPU is clearly a favoured institution. In the annual competition for university entrance, some 7,500 students a year, or roughly ten times as many as the annual entry, put BPU first on their list of desirable universities. So why not grow faster? Fan's reply contains an element of paradox: even an industrially oriented university should not grow too quickly for the health and wellbeing of China's industry. But, secretly, the objective may be to avoid jeopardizing quality in the chase for numbers. □

Plant taxonomy

South China's germ-plasm tank

TRANSPLANT the Royal Botanic Gardens, Kew, from the suburbs of London to a subtropical climate and you would have the South China Institute of Botany, twenty tortuous kilometres from downtown Guangzhou, the provincial capital of Guangdong. But the institute has long since outgrown its role as the taxonomic centre of luxuriant South China, with interests that range from plant ecology to agricultural botany.

The origins of the institute parallel in interesting ways the emergence of modern China from its feudal past. Guangdong was the base from which, in the 1920s, the revolt against the surviving feudal barons was organized by Dr Sun Yat Sen (after whom the university of Guangzhou is named). The province then had the sense of being at the centre of reform in China. For a time, in the 1920s, an island in the Pearl River was the site of the military academy whose students at various times counted among their number not only Mao Tse Tung and Chiang Kai-shek (later the Kuomintang leader) but also Zhou Enlai, the Chinese Prime Minister in the period immediately after the Cultural Revolution.

By 1928, the plant taxonomist Chung, then based at the Sun Yat Sen University, embarked on an ambitious taxonomic study of South China. Over the succeeding twenty years, undisturbed by revolution, invasion (by the Japanese) and civil war, Chung and his team, which never exceeded 26 people, scoured the region for plant specimens, which now make up the core of the institute's herbarium, a vast collection of 600,000 dried specimens. Soon, the institute hopes, the herbarium catalogue will be computerized. And there are plans to transfer the whole of the herbarium to an air-conditioned building.

The present director, Dr Kuo Chun-Yen, has his intellectual roots in the Middle West of the United States; a graduate of the University of Shanghai, he took himself to the University of Chicago for graduate studies in 1947 and was then stranded at the University of Michigan (at East Lansing) because the United States and the new Chinese People's Republic did not establish diplomatic relations with each other until 1955. The result is that Kao bubbles with enthusiasm for the plant-breeding unit that covered the Middle West with hybrid corn in the 1960s. In September this year, he was packing his bags for yet another visit to East Lansing, an extended stay supported by the US National Academy of Sciences.

Kao's interests in agricultural botany reflect the present pattern of the institute's work. The establishment was moved from the university to its present site in 1954, and four years later spawned

the adjacent 30 hectare botanic garden. The subtropical climate has ensured that, in a mere thirty years, traces of the previous army barracks have been obliterated by lush green vegetation. In 1955, the institute also acquired the 1,200 hectare site in the mountains southwest of Guangzhou which has become an arboretum of such distinction that it is likely to be the centre of one of UNESCO's Man and Biosphere projects.

Nationally, the South China Institute is one of three laboratories whose interests between them span the whole taxonomy of Chinese plants (the others are at Shanghai and Beijing). Jointly, the three institutes have embarked on the compilation of a comprehensive Chinese flora, a gigantic undertaking whose timescale remains undefined. But in practice, the institute's interests inevitably centre on the agricultural needs of South China. The techniques of meristem culture have become the spearhead of plant breeding, here as elsewhere in China. Kao is especially pleased with a project, now in the development stage, to propagate a species of begonia growing naturally on rocky surfaces which has been collected almost to extinction on account of its medicinal properties (but which, in any case, makes a pleasing tea-like beverage). Kao emphasizes that the topography of much of China is unsuitable for the growth of paddy rice, for which

reason there is a great need for the development of other kinds of crops. Kao figuratively snaps his fingers with frustration that the Chinese gooseberry has been transmogrified into New Zealand's kiwi fruit. The institute is engaged in the general concern to develop crops that may be grown successfully on the island of Hainan, the strategically important island off the south coast of Guangdong that faces North Vietnam, as well as in what seems to be the national concern that lychee fruits should be prevented from deteriorating during their distribution.

By most yardsticks, the South China Institute is a huge laboratory, with a staff of 560, of whom 260 are scientists with degrees. It is as well equipped as anybody could ask. As at most other laboratories in China, there is a curious contrast between the internal finish of laboratory buildings, which seem unusually to be shabby sources of powdered cement dust, and the spick and span appearance of the instruments, mostly newly imported from overseas.

Like the other major institutes of the Chinese Academy of Sciences, the South China institute is an important centre for the recruitment and training of professional scientists. The competition for a place at the institute is keen, not least because it seems to be generously provided with funds to support travel overseas. For the past six years, the institute has been regularly sending people to work overseas not merely so as to read for PhD degrees at universities in the West but also

Fudan powers nuclear industry

STANDING around president Xie Xide in the photograph below are the senior science staff of Fudan University, one of China's top comprehensive universities. In contrast to nearby Japan, women professors in science faculties are not uncommon.

Fudan University was set up in Shanghai in 1952 in the days when a major first task of staff was to translate Soviet textbooks. Nowadays, most staff have been to study in the United States or Europe and the university is adapting to the new open policies. A new School of Technological Sciences

was set up last year, not to train engineers, but to produce top graduates with the scientific grounding necessary for research and development. Among research in progress is work on oil prospecting software for a homebuilt high-speed computer, zeolite catalysis, control of pests by viruses, and VLSI (very large scale integrated circuit) photoresists. There is also one of the nation's few departments of nuclear science, some of whose graduates will go to the new nuclear power plants now being constructed.

The teaching system is also becoming more liberal with students allowed to choose courses to go with their majors and the introduction of a US-style credit system. There is talk — as in Qinghua University — of an exam at the end of the first year to weed out students who are not making the grade, although it is bound to face opposition. Like universities elsewhere the staff is divided into those who do research, and those who mainly teach with perhaps a third of their time free to research. Rotation between groups takes place, but irregularly. Research facilities have much improved in the past few years with a massive infusion of new imported equipment into the science building.



Left to right: Hua Zhong-yi, vice-president; Zheng Qi-ke, nuclear science; Wang Li-hui, materials science; Xie Xide, president; Wu Li-de, computer science; Su De-ming, biology department; Zhou Dun-ren, foreign affairs; and Gao Zi, chemistry department and recently at Imperial College, London.

to work on collaborative projects. As part of a collaboration with the University of Utah on photosynthesis under the auspices of the Carnegie Institute of Washington, for example, Kao has two of his people at Salt Lake City on a five-year stint.

Reform has also begun to make its mark. The research programme at the institute is being formally evaluated by a committee including people from outside the institute, and a process has been set up for assessing the performance of individual researchers so that, over the years, people can be persuaded that their personal interests coincide with the national interest that the scientific labour force should be more mobile.

As elsewhere, the Cultural Revolution is philosophically regarded as a phenomenon belonging to the past. Kao and many of his staff were sent off to the countryside at the beginning of the upheaval, but he says that after a little while, they were able to move some of their equipment into exile as well, and get on with their work. During the period, they were able to produce three volumes of advice for barefoot doctors on the medicinal use of Chinese plants, as well as giving some of the peasants with whom they worked first-hand instruction on the use of new techniques and novel cultivars. And even now, on a strictly voluntary basis, the laboratory thinks it worthwhile that some of its scientists should work closely with peasants in the countryside, providing some of the services of what would elsewhere be known as agricultural extension services.

Guangzhou's botanic garden is the creation of an Edinburgh-trained agronomist, Professor Chen Fung Huai, now 85 and living in retirement in the grounds of the South China Institute. Chen is now a frail shadow of the man who, in the period after 1949, seems to have made it his personal mission to scatter botanic gardens about China.

Botanic gardens, he says, are not parks or playgrounds, but "open-air laboratories". Each new site is a different challenge. People have to worry not simply about the climate but the pH of the soil, and must be able to guess how the microclimate of each part of a new garden will change as it matures. During his career, Chen has founded botanic gardens in places such as Beijing and Nankin.

Chen is now the South China Institute's most distinguished resident. Kao, the director, says that he has to take care to preserve Chen's energy, protecting him from visitors whenever possible. It is one mark of the way in which ties between China and the rest of the world have survived the upheavals of the past half-century that it should be possible to have a polite conversation about the temper of a Scottish university in the 1930s in such a faraway place as this suburb of Guangzhou. □

Guangdong

The centre is a long way away

SOUTH China is as different from North China as is, say, Louisiana from Massachusetts. The topography, to be sure, may look much the same, but heat and humidity give both southern regions a distinctive sense of being different. But there is another influence in South China — Hong Kong and, to a lesser extent, Macao. The free port ("special economic zone") surrounding what are called, in Hong Kong, the New Territories is the most successful of the four now operating, and has acted like a magnet in shaping the direction of economic activity in the province of Guangdong. One curious sign of the proximity of Hong Kong (about 100 km away) is that it has been for the past year permissible for people in the province to watch Hong Kong television programmes (for which purpose an imported receiver is required). There can hardly be any greater contrast than that between the staid Chinese channels, which broadcast Party meetings, accounts of industrial improvement and scenes from well-ordered family life, and the steady output of violence by video that distinguishes the Hong Kong stations.

Guangzhou, the provincial capital, is also the seat of a branch of the Chinese Academy of Sciences, a distinction that is somewhere in between an administrative decentralizing step and a means of recognizing the importance of a provincial centre. But the Guangzhou branch differs from that in, say, Shanghai in that its relationship with the provincial government is close. Formally, there is a Guangdong Academy of Sciences which is directly responsible for the seven provincially supported research institutes, and which manages the local branch of the national academy as well.



With travel restrictions inside China eased, the Great Wall is always full of tourists from all over the country.

Thus the local branch is a kind of Cantonese contract manager of the six institutes of the Chinese Academy of Sciences in the locality (oceanography, geology, chemistry, electronic technology and energy conversion as well as the South China Institute of Botany — see opposite). The clutch of institutes financed by the provincial government, in fields such as geography and microbiology, serve largely provincial needs; officials are proud that, in the past few years, the attention the microbiologists have paid to the improvement of mushroom strains has, for example, made it possible to increase the income of peasant mushroom growers by 10 million yuan a year. Another proof of Guangdong's capacity to look after itself is that the Academy seems to have been able to open a new research institute, that for electronic technology, at the height of the Cultural Revolution.

For all its operations, the Guangdong Academy gets 25 million yuan a year from the Beijing Academy and 7 million yuan from the provincial government. Officials of the local academy are enthusiastic supporters of the government's policy of modernization. They see it as one of their principal tasks to persuade more scientists into industrial employment, to which end they are doing everything they can to have their own institutes work more closely with industrial partners.

The Guangdong management is plainly not informed of every detail of what its institutes are up to in these fields, however — the central office had not learned in late September that its institute of electronic technology had sold a software package to a Hong Kong company for HK\$100,000. But that is entirely within the rules. Only if the earnings of individual institutes became a large proportion of their budgets would it be necessary to consider whether the income should be used to help finance other institutes.

Coping with growth may be Guangdong's only source of serious worry. For the time being, the officials say, there is enough money to keep the institutes hard at work. The supply of people is sufficient, but there is some concern about the quality of those leaving the universities. Equipment is no great problem now that the institutes with research reputations can get their hands on foreign exchange.

So should not something be done to improve the universities, and to increase their intake of students? Here again, Guangzhou echoes Beijing. For the time being, there can be no substantial increase of the university population. The immediate hope is in continuing education, either at the workplace or by private study, with help provided by educational television programmes. □

Shanghai biology

Largest city looks to itself

SHANGHAI, China's largest city (on the strength of its ports and industry), has every reason to regard Beijing, China's capital, as an administrative centre somewhere to the north. The relationship between the two cities resembles, for example, that between New York and Washington DC, or, in the nineteenth century, between Manchester (England) and metropolitan London. This is the spirit in which Shanghai takes pride in being one step ahead of the capital. Institutes of the Chinese Academy of Sciences at Shanghai have thus been first in the field with the synthesis of insulin, and have designed China's indigenous nuclear reactor.

Another sign of the way the wind is blowing is that the local branch of the Chinese Academy of Sciences is now busily welding together on a single campus a clutch of biological institutes that, between them, will have more than 3,000 qualified people on their books. The intellectual architect of this scheme is Professor Cao Tianqin, now the president of the Academy's Shanghai branch and director of its division of biological sciences.

Apart from the biochemistry institute (of which Cao was the director until a few years ago), there are institutes of physiology (housed in a Victorian gothic mansion), organic chemistry (oriented towards natural products of medicinal value), materia medica (more so), cell biology, brain research, plant physiology and entomology. The laboratories are united into a kind of common purpose, not the least by an ambition to be doing today what Beijing may do tomorrow.

The Shanghai institutes are especially proud of their overseas connections. Cao took his PhD at Cambridge. Professor Mei Zhen-Tong, the woman who is director of the Institute of Physiology, is similarly trained overseas. Professor Chang, who founded the brain research institute by fission from physiology in 1981, had returned from a distinguished career at the Rockefeller Institute, New York, and at John Hopkins. The Australian connection is also conspicuous at Shanghai, especially in plant physiology and entomology.

This complex of laboratories is splendidly equipped. During the past few years, there seems to have been very little restraint on the supply of hard currency for the purchase of equipment, so much so that there are occasional signs that some of what has been bought in from overseas has not yet been used for its intended purpose. One mark of the state of the equipment at the Shanghai institutes is that the important suppliers from the United States and Europe have thought it worthwhile setting up agencies in the city.

Although most people in these biological laboratories recognize that the future lies with molecular biology and its applica-

tions, for the time being there are two serious impediments. First, in spite of many efforts during the past few years, plans to provide a central agency for the supply of essential reagents such as restriction enzymes have not succeeded; the purity and activity of the indigenous products are variable, so that too many researchers and their helpers must spend precious time making their own. Much the same applies to the supply of radioactive chemicals. The specific activity of reagents labelled with active carbon is never great enough to satisfy the molecular biologists.

Meanwhile, there is no shortage of interesting work on hand. The organic chemistry institute, with a total of 1,200 members of staff (500 or so employed on pilot-plant work) spread their interests from the role of nickel and molybdenum in catalysis to the search for medicines. One such success is the characterization and purification of an abortifacient in a traditional medicine which consists of a polypeptide with 230 amino acids. In the pure form, microgram quantities of the material appear to be effective. The laboratory itself is manufacturing the material for distribution in China, and estimates that more than one million women have so far

Acupuncture boom punctured

ACUPUNCTURE is still widely used in China for the treatment of various ailments, rheumatism, for example. But there now seems to be a retreat from the assertion, common during the Cultural Revolution and believed by many Western physicians at the time, that acupuncture can be an effective analgesic in surgical operations.

Dr Jiang Zhen-yu (C.Y. Chiang), from the Shanghai Institute of Physiology, is judiciously cautious on the present use of acupuncture. He says that the ministry of health has put out a decree restricting the analgesic use of acupuncture to operations on the thyroid, the diaphragm and similar procedures. The general use of anaesthetic acupuncture has otherwise fallen into disuse. And there is no means of telling how widely practised are the uses allowed by the government decree.

Jiang insists that the new scepticism does not extend to the therapeutic use of acupuncture. In saying that, he reflects one of the most striking features of Chinese health care to visitors from abroad: the apparently easy coexistence of scientific and traditional medicine. The explanation, to scientist apologists, is that traditional medicine will be found to be just as objective when biochemists have identified the active principles of traditional remedies, dried bears' gall-bladder (a treatment for kidney stones), for example. □

made use of it. The obvious next step is to tell which parts of the molecule are essential for the biological activity in the hope that synthetic production will be possible. Similarly, there is a continuing hunt for antimalarials; the active principle of the *Qinghan* species, identified at the laboratory in 1979, has now been synthesized.

Between them, the Shanghai biology and biochemistry laboratories span a vast range of interests, from neurophysiology and the search for new drugs to classical environmental physiology.

The laboratories also have a crucial educational role. The organic chemistry institute alone has 150 graduate students, and is now selecting 6 or 7 MSc graduates a year for its PhD programme. With the help of imported computer equipment, the laboratory is also setting itself up as a national centre for chemical data.

Most people in this Shanghai complex think they will do well out of the reform. The good basic research people, they say, will succeed in the competition for research grants from the National Science Foundation, to come into being next year, while those whose work has practical applications (the cell biology institute, for example, is strong on the endocrinology of fish reproduction) will also thrive.

The cell biology institute hopes, under the reform, to become an "open laboratory", a distinction that would allow it to take in researchers temporarily from university departments, to be more free than at present to spend funds on overseas travel and on inviting visitors from overseas. (Already it has a suite of laboratories, paid for by the West German Humboldt and Thuysen foundations, which houses two West German doctoral scientists in spectacular luxury by Chinese standards.) But greater freedom in the selection of graduate students is ultimately the prize.

Under the new system, will it be possible to safeguard the interests of basic research? Most of the laboratory managers are conscious of the need, even when they add that China's industry is too short of skilled help. The most obvious danger is that even when people succeed in the competition for research grants from the National Science Foundation, their salaries will remain what they are at present, while there is at least a chance that colleagues who work on industrial contracts will find that their personal rewards are substantially greater than their salaries. Given the resentment about the poor rewards of research now building up in the laboratories, laboratory managers will be tempted to share outside income on some egalitarian basis. But even so, laboratories concerned primarily with basic research will be at a disadvantage. Nevertheless, the president of the Academy of Sciences, Professor Lu Jiaxi, says in Beijing that "they'll have to wait another five years" (until the next five-year plan) to share in China's growing prosperity. □

High-energy physics

Towards home-grown mesons

WHY should a country such as China be building a particle accelerator when countries such as Britain wonder whether they can continue to belong to international organizations such as CERN, the European high-energy physics laboratory at Geneva?

The answers are various. The technology of accelerating charged particles is valuable in itself. Worthwhile accomplishments in such a field are a necessary price to pay for collaboration with others. And in any case, as the economy of China grows to match those of the large industrial nations, it will be natural that China should be in high-energy physics.

But the clinching argument is probably similar to that which sustained high-energy physics in Britain and France in the 1960s; there is a group of Chinese physicists with ability and political influence who consider that China without an accelerator would be a contradiction in terms. Even though the ambitions of the period immediately after the Cultural Revolution have been scaled down, the government is generously backing construction of a 2 GeV electron-positron collider.

The result is the large elliptical hole in the ground now taking shape at the Institute of High-Energy Physics at Beijing. By 1988, this will have been equipped with the appropriate vacuum chamber and magnets, within which there will circulate two oppositely directed beams of electrons and positrons, each beam with an energy of 2.2 GeV.

In these terms, BEPC (for Beijing Electron-Positron Collider) will be most closely comparable to the electron-positron collider called SPEAR II, which operates at Stanford but the design group hopes to attain a luminosity (collisions per square centimetre per second in the colliding region) greater by at least an order of magnitude (specifically $1.7 \times 10^{30} \text{ cm}^{-2} \text{ s}^{-1}$).

This, of course, is both a far cry from the energy of machines such as LEP (being built at CERN), which should give electron and positron beams of 86 GeV with the possibility of an extension to 135 GeV per beam. It is also a far cry from the plans announced at the great national meeting on science and technology in November 1978, when the ambition was to build a machine for accelerating protons to 50 GeV (a more powerful version of CERN's super-synchrotron, say).

That was the goal as recently as four years ago, but detailed design work showed, according to vice-director Zhang Houying, that the cost had been underestimated by 1,000 million yuan or so. The machine now being built is a utility electron collider; the energy of each beam can be tuned between 2.2 and 2.8 GeV, but the option of extending the beam energy to 4.7 GeV has been dropped. To begin

with there will be only one point on the storage ring at which electron-positron collisions will be induced (with provision for a second colliding zone to be added later) and the storage ring will be equipped with an oscillating magnetic field to generate synchrotron radiation with usable flux in the keV range.

One relic of the heart-searching of the past few years is a 35 MeV linear accelerator for protons, built almost entirely from Chinese components, which is being ingeniously converted into a means of generating fast neutrons (for the treatment of cancer patients) and short-lived isotopes.

BEPC itself is the result of a government decision at the highest level (the



Beijing's storage ring takes shape.

State Council). The cost of the construction phase (240 million yuan) is being borne directly by the government even though the institute is an integral part of the Chinese Academy of Sciences' network. ("We couldn't afford it", said one Academy official.) According to vice-director Zhang, the eventual running cost of the new machine will be some 20 million yuan a year; it is still not clear which part of the government will meet the cost, but the Academy seems determined that it should be the government direct.

Since the ground-breaking ceremony in October 1984, work has pushed ahead quickly. The 201-metre tunnel for the injecting linear accelerator (which will produce alternately electrons and positrons at 1.1 GeV) is almost complete, 8 metres below ground level. Concrete is being poured for the containment walls of the tunnel to house the storage ring. Construction should be complete by the end of 1986, with operation 18 months later.

Intrinsic interest apart, BEPC is a marvellous illustration of the problems of building a big high-technology machine in China. Part of the objective has been to

demonstrate, for self-confidence if no other purpose, that the design can indeed be carried through; so the control system (which detects the positions of the electron and positron beams, adjusting the magnetic fields to keep the beams on course) has been elaborately designed around a network of novel Intel integrated circuits.

But as yet, the design is unfinished; last month, no fewer than 15 people from Beijing were working at the Stanford Linear Accelerator Center on the details of a design that will, with luck, incorporate the latest in accelerator technology.

The physical components of the accelerator are more teasing problems. According to Zhang, the sections of the aluminium vacuum chamber are being made in the United States because they are cheaper that way (\$50,000 against 700,000 yuan). The same applies to electronic components, which are 1,000 times as expensive in China as in the United States.

On the other hand, the klystrons that will generate the microwave power to accelerate electrons in the linear injector section are to be made in China because their export from the United States is forbidden; the high-energy physics institute has had to rely instead on the Ministry of Electronics to commission the manufacture of the 16 klystrons required for the linear accelerator (peak power output, 15–20 MW at 2.856 GHz), no doubt to the benefit of China's defence industries.

The US government has nevertheless relaxed the strategic embargo to allow the purchase of a VAX 11/830 computer after 18 months of negotiations, but in return for a promise that the machine will be used only for the collection and processing of data from the drift chamber detector being built for the sole experimental section of the storage ring.

What will the machine accomplish? Some physics, without doubt; even by 1988, the products of electron-positron collisions with total energy between 4 and 5 GeV will not fully have been worked out. There will be plenty of charmed mesons and the institute intends to concentrate on careful measurements of the properties of these particles and the details of the decay of the heavier analogue of the electron and muon (the tauon).

The more enduring objective is to show that China can build an accelerator, and in the process win access for Chinese physicists to more powerful machines being built elsewhere. Already there are members of the institute working at Brookhaven and the National Accelerator Laboratory (Fermilab) in the United States, as well as at Stanford and CERN. The institute also hopes that its new machine will become a focus for collaboration with other developing countries.

For reasons such as these, the institute seems not to be under serious pressure from the government to be more directly

involved with industrial development, some of which is in any case stimulated by the need to develop novel components for the accelerator. With more than 2,000 people (1,200 of them scientists and engineers), the institute hopes to have more than 100 graduate students in the year ahead and a quota of 10 postdoctoral fellowships a year, chiefly as a means of holding onto those returning from abroad.

Curiously, the institute seems to have been spared the worst excesses of the Cultural Revolution, although people like vice-director Zhang (an electrical engineer) spent much of the time as a member of a cadre work-school laying electric-

ity power lines. Its partial immunity seems to have stemmed from a Maoist dictum that it is important to "keep on dividing the constituents of matter" as well as from its antecedents: the institute is an offshoot of the Institute of Modern Physics established in Beijing in 1950, from which has also sprung the Institute of Atomic Energy (in the southwestern suburbs of Beijing) and, less directly, the Shanghai Institute of Nuclear Energy (responsible for the design of the 300 MW indigenous reactor) and the Lanzhou Institute of Modern Physics with a programme of low-energy nuclear physics. The Moscow Institute of Physical Problems is the closest analogue.

China has followed the Soviet Union in its organization.

But China's special claim on attention, according to Zhang Jiqing, is the development of techniques for recovering satellites from orbit (by means of a retrorocket and a parachute) and the use of a single rocket for launching several satellites. There is nothing remarkable about the sequence in which the rockets at the ministry's disposal have been developed. The first was a three-stage affair, with two liquid-fuel stages and a solid-fuel booster on the top.

The workhorse of China's space programme until recently, Longmarch II, had two liquid stages, and has been used on nine occasions, with only one failure right at the beginning. Longmarch III, according to Zhang, has three stages, of which the uppermost is fuelled by hydrogen and oxygen; he says that it is comparable with the first version of Ariane 1 in that it should be capable of putting 1 tonne of mass into a low orbit in preparation for putting part of the mass into geosynchronous orbit. Improvements now being undertaken, Zhang says, will increase the masses by a half, so as to put 1 tonne, typically the weight of a communications satellite, where it can be useful.

The scientific programme has concentrated so far mainly on the measurement of energetic particles and cosmic rays. Chinese researchers with plans for satellites must first convince their institutes of the good sense of their proposals, whereupon the institute and its parent agency will seek to negotiate launching space.

Plainly, this process is as tortuous in China as elsewhere. Thus one of the boasts of the Guangzhou Institute of Electronics is that it has already finished work on the design and construction of the data acquisition kit intended for an astronomy satellite "soon" to be launched, and that it has already sent the equipment to Beijing.

Space programme

Next, the non-stick heat pipe?

CHINA in space is not the joke suggested by its first satellite, launched on 24 April 1970, and still affectionately remembered for its broadcasts (audio, not video) of a folk song. Indeed, there are no doubt many who would consider that the rapid development of China's rocketry is almost the opposite of a joke.

On the civilian side, China has since 1970 launched a total of 16 satellites. Last year (8 April) the Ministry of Astronautics succeeded in putting a small satellite into a geosynchronous orbit, preparing the way for the series of telecommunications and broadcasting satellites scheduled for next year and thereafter.

Indeed, the ministry is now talking up the possibility of selling launching facilities, just as do the managers of the Ariane and US shuttle programmes. Zhang Jiqing, an engineer with the ministry and director-general of what is called the Foreign Affairs Bureau, says that there have been conversations about satellite launchings with the Netherlands, Sweden and even the British government (represented by the Rutherford Appleton Laboratory of the Science and Engineering Research Council, a grant-making agency). But nothing has yet been fixed.

The ministry says its interests are entirely civil, but it goes without saying that the civil space programme is an outgrowth of the military programme on whose behalf patches of the Pacific Ocean are from time to time declared out of bounds to shipping so that a military rocket may plump down without doing damage.

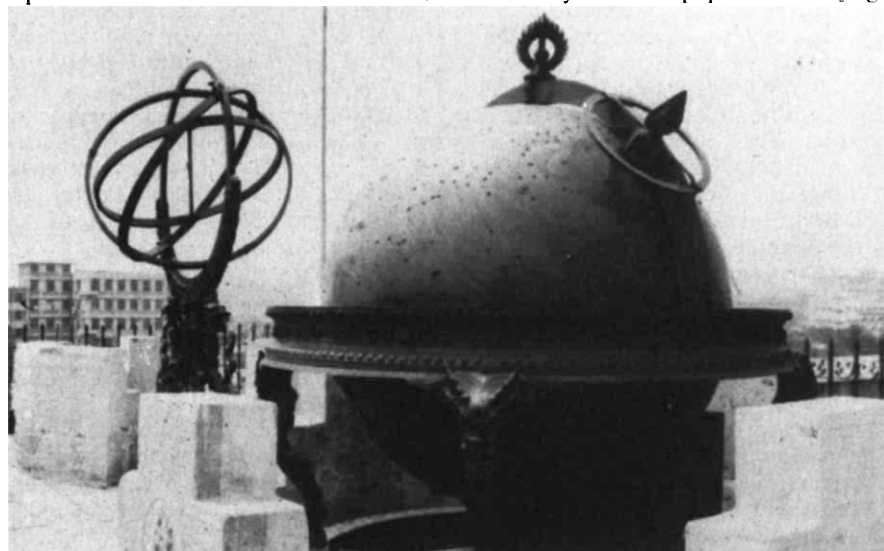
Indeed, Zhang's simple answer to the simple question of what a developing country such as China is doing in space is that the country has a need (for telecommunications and broadcasting satellites especially) and also the means. The liquid-fuelled rockets (using unsymmetrical dimethyl hydrazine as fuel, burned with liquid oxygen) have been labelled sequentially as "Longmarch" I, II and III, with IV and V under development. They have their roots in a series of developments thirty years ago, when the military rocket

programme was begun.

There is a host of research institutes catering for the needs of the military and civil space programmes, many of them the property of the Ministry of Astronautics, others operated by the Chinese Academy of Space Technology, which is itself influenced (and partly financed by) the National Commission for Science and Technology for National Defence, constitutionally similar to the civilian academy on which the Chinese Academy is dependent.

There are special research institutes in fields such as microelectronics, but the design and fabrication of launch vehicles is the responsibility of an industrial organization, the Wangyuan Industrial Corporation.

There is no reason to doubt what people such as Zhang say about the civilian character of his ministry, which seems to have been hewn out of the defence establishment, and given a separate identity, so as to allow military development to continue in isolation — and secrecy. In this diligent separation of the two faces of research,



A sextant, quadrant and celestial globe built by the Jesuits for the Qing emperors are on display at the Ancient Beijing Observatory, a massive gate that was once part of the city walls. Kublai Khan built the first observatory here in 1296. More recently, some extra "stars" were added to the celestial globe when it was pierced by Japanese bullets during the occupation of China.

Zhang, speaking for the Ministry of Astronautics, says that he knows of no plans to launch such a satellite.

Inevitably, the ministry employs technical people on a huge scale. Zhang estimates that its technical staff numbers 20,000, perhaps a quarter of them with university degrees. The ministry seems to have first claim on the output of graduates from a number of university departments as well as its own specialized college.

The ministry seems well aware that its chances of winning business in the market for launching satellites will turn crucially on its domestic success, whence the importance attached to the launching of a full-scale but still experimental telecommunications satellite early next year. The first television broadcasting satellite is now being built, but will not be launched until 1987 or 1988. There are also plans for meteorological and Earth resources satellites.

So is all this effort worthwhile, at least

at this early stage in the industrial development of China? One important spin-off, according to Zhang, has been that China has been required to set up factories for the manufacture of the special materials needed in the space programme, and in the process has acquired novel technology. The fly in this ointment is that many of the factories built for this purpose have been isolated from other general-purpose enterprises, but Zhang thinks that may change now that the government is doing everything it can to encourage mobility within the economic system.

Asked for a specific example of an innovation within the space programme that had found more general application, Zhang (after some thought) offered the case of the heat pipe (a way of transferring large amounts of heat by means of liquid metals), which is slightly reminiscent of the US NASA boast in the 1960s that the space programme had given the world the non-stick frying-pan. □



The aperture synthesis metre-wave radio telescope at Miyun, outside Beijing.

will soon have a station. A 25-m antenna facility for the 1–20 cm waveband which can link up to the world network is almost complete at Shanghai. As the only station in this part of the world it will be highly valued.

Solar studies, another relatively inexpensive area, and of practical use in predicting magnetic storms which disrupt telecommunications, are also well developed, at least in the more classical areas. New equipment coming into service includes a very fast recording solar heliometer telescope at Beijing University. A new telescope for observation of the fine-structure of the Sun is now complete at Yunnan Observatory and another will come into operation at Nanjing Observatory next year. Several major expeditions to observe eclipses have also been mounted and made good use of the unique observing conditions available in the Himalaya and Karakorum mountain ranges.

A unique site — in the Chaidamu Basin in the Gobi desert — has also been found for the new millimetre-wave telescope. It is quite high (3,000 m), flat (which makes it easy to do interferometry and to build roads) and very cold. That means there is very little water vapour in the atmosphere which makes for much higher resolution than comparable instruments at sea level. A 13.6-mm imported telescope will be run by the Purple Mountain Observatory and should be in operation next year. Here China can make major contributions for many molecular lines remain unexplored.

An attempt to fill an empty niche is also being made with the Miyun radio telescope. A set of antennas used for solar interferometry has been expanded into an aperture-synthesis array operating in the metre waveband (232 megahertz). Expensive and sophisticated centimetre-wave telescopes are already in place in the United States and United Kingdom so the telescope was designed for wavelengths they will not reach. Nor will the very large arrays now being built come to these wavelengths so Miyun should provide unique data for years to come. At present a two-year survey of the whole northern sky is under way. Any new variable stars, stars of unusual spectral type and the like that turn up among the 100,000 or so sources expected to be present will be studied in more detail later. □

Astronomy

Things could be looking up

NEW astronomical facilities are appearing one after another in China: completed this or last year are a metre-wave and two solar telescopes; and nearly complete are a very long baseline interferometry (VLBI) station, a millimetre-wave telescope, a 1.2-m infrared telescope, and a 2-m optical telescope. But it would be an oversimplification to say that Chinese astronomy is booming.

As Professor Wang Shouguan, director of Beijing Observatory explained, the apparent burst of activity is more a matter of the Cultural Revolution having synchronized many projects. It was back in 1957 that a large optical telescope was first planned, for example. But then the Cultural Revolution came along and different parts of the telescope were slowly built at different places. Engineers are now just completing the daunting task of remodeling the parts to fit them together (which is "worse than doing it from new") and then the telescope — a 2.16-m reflector — will be installed near Beijing next year.

Major observatories have been built at Beijing, Purple Mountain (Nanjing), Shanghai, Yunnan, and Shaanxi (a relatively new time-service observatory) with the largest optical facility a 1-m telescope at Yunnan. But a new 1.2-m infrared telescope will be in operation at Beijing Observatory by the end of the year. Undergraduate courses in astronomy are given at Nanjing, Beijing Normal and Beijing universities and the Academy's University of Science and Technology at Hefei has an astrophysics centre for graduates.

Overall, China's astronomical facilities are quite modest. But that is not necessarily a bad thing. Professor Wang sees the immediate aim as training a corps of young

scientists, and for that small to medium-sized facilities are excellent because you have to "think hard to get the most out of them". When Chinese astronomers are more sophisticated they can try some really big projects. And even if more money were available Professor Wang's own view is that it should go on laboratories, computers and young people rather than big telescopes. That being said, China's facilities are really quite advanced for a developing country.



Professor Wang Shouguan, director of the Beijing Observatory.

High-level research has been carried out in time-service and positional astronomy where observing equipment is not so expensive and results of practical use can be obtained. Classical optical methods have been improved and China was first to develop an automatic photoelectric version of the Danjon impersonal astrolabe which truly eliminated observer error. Also on the drawing board (and attracting worldwide attention) is a 1.5-m astrometric reflecting telescope for the Shanghai Observatory. This exceptionally large telescope for astrometric work would enable the proper motion of faint stars to be studied photographically.

Much of such classical work is now being supplanted by VLBI and China too

Earthquake prediction

The calm before the storm?

A FEW weeks ago, with reports of the earthquake that killed more than 5,000 people in Mexico City still fresh, Professor Ding Guoyu of the State Seismology Bureau at Beijing confessed himself a worried man. The bureau and its Centre for Analysis and Prediction seems persuaded that there are alternating periods of seismicity (10–12 years long) and of quiescence in the occurrence of major earthquakes in China, and that a new period of activity is about to begin.

Notoriously, the period of the Cultural Revolution was marred by natural disasters, among which earthquakes were prominent, culminating in the loss of 240,000 lives in the earthquake near Tangshan in July 1976. Since then, Professor Ma Zongjim says, there was a period during which earthquakes of magnitude greater than 6.5 on the Richter scale were absent from China until this August's earthquake in Xinjiang province. So now there is a hurry to complete the network of seismic stations being thrown across China under the aegis of the bureau.

The generosity of the government's support for the bureau (with a budget of 100 million yuan a year) is conspicuous. The seismicity of China has two roots, the subduction of oceanic plates beneath the Pacific rim and the stresses and subse-

quent strains of the Earth's crust which are consequences of the collision between India and Asia.

Ding points out that China differs from seismic regions such as Japan in that a large proportion of its earthquakes are within the continental crust (and not buried in some oceanic trench as off the coast of Mexico). That continental earthquakes tend to be exceptionally damaging is easily understood, and is borne out by the evidence that four major earthquakes since 1932 have killed more than 750,000 people.

Accordingly, the plan is to cover China with a network of seismic stations whose function will be systematically to record smaller earthquakes in the hope that these will somehow be pointers (or forerunners of) catastrophe. There are four separate research institutes within the bureau, with its headquarters in Beijing, and a total of 24 provincial seismic bureaux intended to provide operational advice for provincial governments.

Although there are 400 seismic stations of various kinds in China, only 20 are at present equipped with standardized instruments, plus extensometers and tiltmeters. (The stations are also charged with collecting geomagnetic data, raw material for study of the secular variation of the

geomagnetic field.)

Altogether, the bureau has a staff of 5,000 technical people, recruited from university departments of geology and geophysics as well as from its own training school. Its managers say that the network they are developing cannot afford to omit any substantial part of China.

Ma, for example, points out that the earthquake of 1920 which killed 180,000 people had a Richter magnitude of 8.5 and yet occurred in a region which has since been apparently quiescent. It would be dangerous, the argument goes, to neglect such a region simply on the grounds that nothing much has happened in 65 years.

For the time being, the bureau is more an expression of anxiety about a recurring serious problem than a testimony to special Chinese expertise in earthquake prediction. The bureau, originally one of the Chinese Academy's institutes, was made independent on the recommendation of the State Council in 1980. It has to manufacture its own instruments (at a factory near Beijing) and is conscious that these are neither as sensitive nor as accurate as the US instruments being operated at Wuhan under the terms of a collaboration agreement with the United States.

The feasibility of earthquake prediction remains untested. The bureau says that when the immediate task of completing the seismic network is finished, it will try out all possible methods, including the use of accurate triangulation to monitor the movement of active faults. But during the recent quiescent period, the bureau has been looking for trends that may be meaningful in the historical records, which cover the past 2,000 years in reasonable detail.

As the world knows, there is one spectacular success on record for those who would predict earthquakes — the successful prediction of the earthquake near the city of Shenyang in the northern province of Liaoning, abutting the Korean border. This appears to have been an exceptional event, with a whole string of moderately sized earthquakes during the preceding two months converging on what eventually turned out to be the epicentre (see panel on left). Even so, the bureau can and does claim credit for avoiding the loss of more than 100,000 lives.

Just how, and when, the bureau will fulfil its mission of predicting a substantial proportion of the major earthquakes of China remains to be seen. But it works with the Institute of Soil Mechanics (of the Chinese Academy) on the design of buildings that may more adequately withstand major earthquakes. As in other fields, people are eager to learn whatever can be gleaned from other people's experience, to which end no fewer than 20 of the bureau's researchers will be at the forthcoming symposium on prediction, to be part of the annual meeting of the American Geophysical Union next month (December 1985) in San Francisco. □

Portents read in nick of time

THE circumstances of the Shenyang earthquake of January, which eventually was of magnitude 7.0, are unusual to say the least. The Qiang Shan peninsula, which forms the eastern boundary of the Bohai Sea, lies immediately to the east of an active fault running inland towards Shenyang. It is intersected almost at right angles by another, cutting across the base of the peninsula and the broad belt of thick sedimentary deposits supporting, among other things, the valley of the River Hun.

According to Professors Ding and Ma, the most conspicuous of the objective signs of trouble ahead were the small earthquakes preceding the magnitude 7.0 event by as much as two months. There were no fewer than 500 of these premonitory earthquakes in the five days before the major event, all of them within a narrow area centred on the eventual epicentre.

Other objective forewarnings included the variation of water levels in wells and dramatic changes in the output of oil from the oil wells drilled in the neighbouring sedimentary basin, which is a substantial source of petroleum.

There were also reports of strange animal behaviour, including the discovery that overwintering snails that would normally have stayed in hibernation were found to have ventured out and to have

been frozen to death and of deer that broke their legs by jumping fences too high for them. The obvious difficulty is that these abnormal behaviours, if accurately recorded, are as likely to be a consequence of the foreshocks as a sign that animals have a hidden sense of earthquakes on the way.

The Bureau of Seismology does not itself pretend to have an explanation of these phenomena, but says it has asked the Institute of Biophysics (under contract) to look into the question. The institute has a serious interest in neurophysiology, and seems a little sheepish about the whole affair, saying it will have to understand the animal nervous system much more fully before it can give a helpful answer.

No amount of scepticism on these points can rob the seismological bureau of the credit for having predicted an earthquake. But the procedure is far from simple. The bureau says that it does not itself have authority to publish earthquake warnings, only a provincial governor can take that responsibility. In January 1975, the provincial government was persuaded of the significance of the forewarning shocks by 10 a.m. one morning, eighteen hours before the earthquake finally came. It was a narrow squeak not merely for 100,000 people but for the reputations of the provincial government and the bureau. □

Cultural Revolution

True horror stories

ALMOST all those now working as scientists in China are people whose careers were launched before the horror of the Cultural Revolution began. Since the "smashing of the Gang of Four" in 1977, there has only just been time for a trickle of people to work their way through an undergraduate course, and through a PhD course on top of that, so as to become established in a job. The problem of "the missing generation" is apparent, and its implications widely discussed. But what happened to those now at work during that dreadful time? Sheer curiosity impels the question; politeness implies that an answer is usually forthcoming. Here are some case histories.

- One plant scientist spent a year working as a peasant in the fields 200 km away from the laboratory, a further year designing simple experiments to demonstrate the relative benefits of different ways of growing crops and then the whole of two years working on laboratory research with equipment transported from the main laboratory with the help of the "coordinating committee" set up in Guangdong by the provincial government.

- A mathematician (again in Guangdong) who had worked in the field of operational research spent the time of the Cultural Revolution working on the "optimization of industrial production" while travelling around factories in the region.

- An electrical engineer turned high-energy physicist worked on a project for constructing electricity supply lines.

- One mycologist was encouraged to spend several years scouring China for species and strains of soil fungi that might turn out to be sources of previously unknown antibiotics.

- A biochemist was allowed to spend the period of the revolution screening 20,000 people for the presence of alpha-feto-protein in their blood plasma, on the supposition that this might be diagnostic of the liver cancer (linked with hepatitis B infection) which is common along the eastern seaboard of China.

- One of China's most distinguished scientists, Professor Cao Tianqin, president of the Shanghai branch of the Chinese Academy of Sciences who, as director of the Institute of Biochemistry at Shanghai, organized the synthesis of insulin twenty years ago, tells the following horror story, without noticeable anger: "One day, they came and arrested me, my wife and my son. On the second day, they imprisoned me in the basement of the laboratory's animal house; for the next three years, my companions were the rats and the guinea pigs. I didn't see my wife during the whole of that time, and they wouldn't tell me how she was. That was especially worrying because I knew she was due to have an operation for cancer of the breasts. Only

after I was released did I learn that she had survived, but that her recovery from the operation had been very risky because the nurses and the physicians were fighting with each other, and had no time to look after the patients properly."

The moral, for those who would survive cultural revolutions anywhere, seems to be that one should at all costs avoid being a person in authority, for then there is no alternative to being labelled an enemy of the people. Those whose research has obvious practical application may, on the other hand, be able to stay in business. Even those whose research is somewhat academic may be able to persuade the revolutionaries of the opposite; appearances, in China's case, seem to have been more important than substance.

After the event, people seem uniformly to be more amused than offended by their experiences. Some even say that they have never been as healthy, and that it is beneficial that intellectuals should have the experience of working shoulder to shoulder with working people. Those who lost their lives because of the Cultural Revolution, are not available for comment.

Yet people do acknowledge that the ex-

perience of this period may have served some lasting purpose. Many agriculturally oriented research institutes are now more acutely aware of the need for what are called, in the West, extension services. And the notion that researchers should work hand in glove with industry, the cornerstone of China's plan for the future, is in the same spirit.

So may not the Cultural Revolution have been a benefit, after all? Faced with that question, an official of the Chinese Academy of Sciences at Guangzhou blurted out that there is nothing wrong in persuading scientists to work alongside peasants, but that sending researchers to the countryside during the Cultural Revolution was offensive "because the object was to wash our brains".

So will the experiment be repeated, or at least might the present opening to the outside be ended? There are three stock answers. One is that it is too soon to tell. Another is that "objective reality", a Marxist term, not peoples' inclinations will determine what happens in China. Yet another, given with much more enthusiasm in the south than the north, is that the process of unwinding has gone so far in the past eight years that it is no longer possible to turn back.

To outsiders, this absorbing question must still be wide open. □

Electronics

Working for industry

THE Institute of Electronic Technology at Guangzhou puts out an advertisement to attract industrial partners that concludes with the exhortation "visit round our institute and have a promising business with us". The message is seriously meant and appears to have been remarkably successful. In September, the institute was boasting that it had been able to sell a software package for the computer manipulation of Chinese characters to a commercial company in Hong Kong for HK\$100,000. One of these days, the product may appear in a Chinese word processor being developed by the Tensing Microprocessor Company, registered in Hong Kong.

The institute is also proud of its origins as a comprehensive research institute established by the provincial government in 1970, when China was in the grip of the Cultural Revolution. Then, it seems, the Guangdong government set up an organization to monitor the effect of the revolution on the infrastructure of its own operations, which is another mark of the sense of independence that flourishes in the south.

The institute became one of those belonging to the Chinese Academy of Sciences only in 1978, with a general remit to develop the techniques of telecommunications and of the applications of computers in this and other fields. With the general

adoption of the policy that government institutes must collaborate closely with industry, the institute has set up a commercial company, the Ling Nan Scientific and Technical Development Company, specifically so as to promote the transfer of technology developed at the institute as well as to provide design and computing services for industrial enterprises in China and elsewhere.

Inevitably, an institute in the field of electronics with Hong Kong on its doorstep must be growing quickly. There are now 400 people at the laboratory, four times as many as at the beginning. Since the institute's incorporation within the Chinese Academy network, much of its work has been stimulated by projects mounted on a national scale by the Academy. Thus the institute is the designer and has become the constructor of the telemetry packages used in the now extensive Chinese programme of balloon-borne physics, the cosmic-ray programme run by the Beijing Institute of High-Energy Physics, for example. Here, as in many other fields, the Chinese have taken great care that they will be able to recover in repairable form any equipment whose construction has been difficult and expensive. This same interest explains why the Guangzhou institute was chosen to build the telemetry and ground station equipment for an astronomy satellite being built by the

Academy institutes, but for which the launching plans are not yet known.

The institute is well along the road the Academy wants as many as possible to follow, that leading to close partnership with industry. Vice-director Chen Yan-yi says that dependence on central funds has declined from 90 per cent of the total budget in 1978 to just about a half now.

For the future, the institute expects that

it will be able to continue growing. Among other things, it is working with the regional government on schemes for the fuller application of microprocessors, not merely within the provincial government's sphere of operations but in Guangdong industry. Hong Kong's influence reaches beyond the special economic zone to the Pearl River, it appears. But the electronic technology institute still has a homespun

air. In a ground-floor laboratory, Wu Caiqing, manager of what is called the Chemical Arts Department of the Ling Nan company, uses what is described as a secret formula to produce etching-like patterns with artistic pretensions on the surfaces of useful and decorative objects of various kinds. Which only goes to show that, in the south, all ways of turning an honest penny are embraced. □

Science popularization

A CAST of thousands

WITH more than a million members, the Chinese Association for Science and Technology (CAST) is beginning to make a mark on the scientific scene. CAST is the umbrella organization for all of China's specialist scientific and technological societies or, as the Chinese would say, it is "a mass multidisciplinary organization of workers in science and technology". The association has only recently re-built its strength after being out of operation throughout the Cultural Revolution.

Those who have attended a conference in China will probably already know CAST for it plays a major role in organizing international exchange. Indeed, the dollars earned from visitors are all ready to help Chinese scientists abroad (when the ministry of finance eases its foreign currency restrictions).

But organizing conferences is only part of CAST's work. Its influence is now felt both by the government and the people. CAST regularly sends its policy recommendations, drawn up by specialist committees from its member societies to Party and government, and at the ministry's request gives technical advice.

CAST also spreads its roots down into local science and technology associations set up to bring science to ordinary peasants and workers. During the Cultural Revolution, there was little opportunity of learning about scientific ideas and CAST itself was disbanded — not until 1980 was it able to hold a national meeting again. But now the press is full of articles about peasants who made fortunes by increasing productivity through application of a little science. Some have achieved virtual stardom: like the chicken raiser in Heilongjiang Province who made so much money that she has been able to build a fifteen-room mansion. CAST is now out in force to take learning into the countryside.

Some campaigns are conducted on an awesome scale. Last year, in Hubei province, 20,000 CAST members went out and gave 80,000 workshops for middle school graduates from farming families — the total audience was five million people.

Keeping up the pace are numerous small scientific associations. At the county level its chief members are likely to be the local doctors, middle-school teachers and technicians from the bureau of agricul-

ture. (Such local improvement societies find their parallel in Victorian England.) Down at the village level (a level of organization formerly occupied by the commune) members will be those who have acquired some special technical skill, say in agriculture or cultivation of fruit trees. Some of their free time is spent in seeing their expertise disseminated. Local halls will be hired for lectures and on a market day a stand may be set up where villagers can learn about improved agricultural techniques.

There are nearly 2,200 science and technology associations at the county level, representing 93 per cent of China's coun-

ties. Some are even able to afford a few full time administrative staff. Down at village and commune level there are 41,000 sub-branches.

CAST does not actually control these lower-level organizations; they are all independent. What CAST does is to prepare publicity material, posters and books, provide speakers from its member societies and help coordinate national campaigns.

On a national scale CAST reaches a massive audience through magazines and books produced by its Science Popularization Press. And for children, there are science summer camps, "love science" campaigns aimed at encouraging kids to read scientific books, fairs showing scientific work by children and a national competition for child inventors. □



Some of the magazines produced by CAST's Science Popularization Press have circulations around ten million. The magazine *Knowledge is Power* (centre) carries some articles close to science fiction — like "Can man live for ever" and "Using hypnosis to stimulate creativity" — but the majority are educational: how electronic devices work, nutrient cycles on Earth, elementary BASIC and the like. Shown behind are regional science and technology newspapers with a more practical bent. They carry articles on such topics as new breeds of fish, forestry policy, how to produce carbon black, and the cross breeding of livestock. Alongside come some features designed to encourage: one story is of a 22 year old high school drop out who develops a new tea variety called "Rainbow" and lands a 7,000 yuan contract.

Aggregation by very large numbers

Computer models of aggregating systems have thrown a useful light on physical processes in the past few years. But too much computer power may make them more intricate than instructive.

THE neater the model, the more easily it is elaborated, and made complicated. That simple truth, always apparent, has been more evident than ever since computer power became more easily and cheaply available. The model of simple harmonic motion represented by the idealized simple pendulum could, no doubt, be made too complicated for a Cray computer to handle by the wooden elaboration of enough complexity.

Much the same, it seems, is now happening to the simple model of diffusion-limited aggregation first put forward four years ago by T. Witten and L.M. Sander (*Phys. Rev. Lett.* **47**, 1400; 1981). The original idea was simple, that of constructing a model to account for processes such as crystal growth whose rate is determined by the diffusion of aggregating particles through solution towards the growing crystal.

The simple version of the model is that in which aggregation takes place on a two-dimensional lattice. Particles are allowed to "diffuse" from the periphery of a piece of the lattice towards a central point by supposing that they move randomly, one lattice step at a time. It is necessary to specify the set of rules that determine when a particle will stick to the growing aggregate; most simply, it can be required to stay where it is whenever it reaches a lattice-site that is immediately adjacent to one already occupied.

Several important demonstrations have been possible with this simple, almost crude, model. Not least, for example, it turns out that aggregates constructed in this way are fractal structures; the numbers of particles they contain increase monotonically with increasing radius, but by some power of the radius which is less than 2.0, the value expected for a structure filling all the dimensions available in a plane. The value of the exponent in the power law with radius is now the familiar fractional dimension. The fact that just such behaviour is observed when dendritic solid structures grow from, say, solution, or when ice crystals form from the vapour, justifies the wide use made of the model and its elaborations in the past few years.

One side benefit of this simple model is that it lends itself easily to computer calculations. Tell the machine the rules, and it will replicate them indefinitely. So it has come about that a small army of ingenious people has been trying variants of the Witten-Sanders model. More than two

dimensions? No problem, except for the cost of computer time. What is the rate at which smaller aggregates will stick together to form larger structures? Again there is no problem once the rules are specified. The fact that the Witten-Sanders model also serves to account for phenomena other than diffusion-limited aggregation, percolation for example, has made it even more popular.

Naturally, there has been a great deal of interest in attempts to make the model realistic. Obviously, in a model growing by random aggregation, there is a possibility that loops will form between one part of the structure and another, thus excluding a large part of the accessible space from occupation. Are these physically realistic conditions, or should they be excluded as rare configurations with extraordinary implications?

Then the simplest set of rules, requiring that particles should stick to an aggregate whenever they reach a neighbouring site, makes no allowance for the likelihood that different sites might be occupied with different affinity. That is why some investigators have set out to calculate the probability that various sites will be occupied by running the random walk backwards.

More recently, attempts have been made to construct still more realistic models of aggregation by changing the rules, allowing a particle either to stick at the first nearest-neighbour site it reaches or, alternatively, to move to a neighbouring occupiable site, perhaps because such a site may be energetically more favourable. (Two neighbours are better than one.) There are reasons to suppose that the results of such calculations may enjoy a degree of realism (see P. Meakin and R. Jullien, *J. Phys.* **46**, 1543, 1985).

The most recent development is an attempt to construct a kind of equilibrium model of aggregation. The idea (due to R. Botet and R. Jullien of the University of Paris-Sud (*Phys. Rev. Lett.* **55**, 1943; 1985) is to begin with an aggregate of some kind and arrange that particles may escape from peripheral sites with single nearest neighbours, wander about by random walking (again on a two-dimensional lattice) and then stick to an aggregation site when next they reach it. If the wandering particle should get too far away, say to the circumference of a distant circle, it will be captured and released again at a random point.

Technically, there is no doubt that the

aggregating particle is stable. The number of particles is essentially constant; the rules specify that there can never be more than one particle on its travels. So what do the calculations show about the way in which the shapes of aggregates change with the repeated release and recapture of particles at the periphery?

First, for a specified number of particles in the initial aggregate, there is a final state with recognizable characteristics determined only by the number. For example, starting with a cluster of 100 particles allowed to randomize themselves by these formal rules, Botet and Jullien show that the radius of gyration of the end result is roughly eight unit lattice spaces. Attenuated structures (say, a string of 100 particles in a straight line) will finish up as a spindly structure. So too will structures that begin as compact clusters, with no spaces between occupied sites.

The obvious disappointment is that the model, as specified, is not a model for aggregates in equilibrium, but instead a model for an aggregate whose size has been arbitrarily fixed. Put simply, it is a model that presupposes that disaggregation is not an option for the system, or that the energy of the attachment of particles to aggregate is infinite. In the circumstances, especially because there can be no attempt to distinguish between the probability with which different kinds of clusters can occur, which is the same as saying that the energy of all configurations is the same. In other words, the model is a long way from being a model of equilibrium aggregation.

That does not, of course, imply that the calculations are without interest. But it is not surprising that the fractal dimensions of aggregates held constant under these conditions should be less than those of the aggregates formed in the first place by the Witten-Sanders process (1.54 against 1.70). Nor is it remarkable that some configurations built from very small aggregates (of, say, eight particles) should occur less frequently than sheer chance dictates. The promised calculation of what happens when larger aggregates split in two, by the severance of a bond chosen at random, may however be more directly relevant to speculation about the way in which aggregates rearrange themselves. The pity is that, for the time being, there are no usable analytical methods of making progress with these problems.

John Maddox

Epstein-Barr virus

Dream or reality of a vaccine?

from A.J. Beale

EPSTEIN-BARR virus (EBV) was first recognized in cultures of Burkitt's lymphoma cells and is a member of the herpes virus family. It was soon established as a cause of infective mononucleosis. There has also been a strong association between the virus, Burkitt's lymphoma and nasopharyngeal carcinoma. Since 1976, Epstein and his colleagues have mounted a systematic search for a vaccine against the virus in the hope that the viral aetiology of the two malignancies could be proved, with much benefit to high-risk populations in Africa, China and South-East Asia. Their studies have reached another landmark, as described on page 287 of this issue of *Nature*.

Epstein *et al.* have previously identified as a candidate immunogen an EBV glycoprotein termed gp340, and have devised means for its extraction, techniques for measurement of both gp340 and antibodies to it, and have developed an animal model of EBV-induced lymphomas in cottontop tamarins (*Saguinus oedipus oedipus*). The stage was set for the critical experiment in which vaccinated and unvaccinated tamarins were challenged with EBV. This experiment and the success of the immunization is now reported.

As the test vaccine, gp340 preparations, containing a minimal amount of residual living virus, were given to two tamarins in eight intraperitoneal doses at fortnightly intervals. Both animals developed antibodies that could be detected by an enzyme-linked immunosorbent assay, by immunofluorescence and by neutralization of EBV transformation of cord blood cells. They were both protected against a massive challenge dose of virus which caused disease and multiple tumours in control animals. Isolated gp340 in liposomes was less immunogenic than the membrane preparation: in a group of four animals given this vaccine, only one produced high concentrations of antibodies and was protected against viral challenge. Thereafter, two animals were given 17 intraperitoneal doses of the liposome preparation at fortnightly intervals and were subsequently challenged. Both animals developed transient inguinal lymph node enlargement after challenge; in one case this was accompanied by transient mesenteric node enlargement.

From the successful development of a vaccine against Marek's disease, to which fowl are particularly prone, it is already clear that a vaccine against tumours caused by herpes viruses is feasible, and the work now being reported on EBV shows that the development of a similar vaccine for a human herpes virus is theoretically possible. Is it likely to be a practical

proposition to make and test such a vaccine? The candidate vaccines of Epstein *et al.* are not practical: clearly, the membrane preparation of gp340 would need rigorous testing to show it was free of infectious virus before its use in man could be contemplated, and either vaccine would need to be presented in such a way that a protective immune response is achieved with fewer doses of vaccine. Doubtless the schedule could be improved to ensure priming and the elicitation of a secondary response.

The most important advance referred to by Epstein and his colleagues is the cloning and sequencing of EBV and identification of the sequences encoding gp340. It should therefore be possible to produce the protein in large quantities. Since it is glycosylated — more than 50 per cent of the mass is carbohydrate — it may prove best to prepare it in mammalian cells, as is done for many other viral immunogens, rather than in bacteria or yeast. Alternatively it could be expressed in vaccinia virus or some other carrier. The groundwork for developing a vaccine against EBV seems to have been soundly laid and the technology to produce sufficient immunogen is at hand.

The major problems of organizing and financing the procurement and testing of such a vaccine remain to be solved. The problems of organizing trials against diseases caused by EBV are formidable, even when compared with other herpes

virus infections. Whereas infectious mononucleosis is an early manifestation of primary infection, Burkitt's lymphoma and nasopharyngeal carcinoma are late manifestations that often take many decades to emerge. No one knows at present whether the late manifestations can be prevented after infection, but current opinion is sceptical. The aim, therefore, must be to prevent infection.

Trials of a vaccine against infectious mononucleosis are a practical and worthwhile proposition, given the will, in Western countries. Proof that EBV infection and this disease can be prevented by a vaccine would warrant the use of a vaccine to prevent Burkitt's lymphoma and nasopharyngeal carcinoma. It is doubtful whether it is practical to carry out a placebo-controlled trial lasting decades, but observation of the effect of vaccine on disease together with more limited trials of the vaccine on viral infections may suffice to demonstrate that EBV is indeed the cause of Burkitt's lymphoma and nasopharyngeal carcinoma.

For a number of diseases, ranging from malaria and pertussis to those caused by hepatitis B and EB viruses, there are now prospects for control by immunization, based on a molecular understanding of the immunogen required to produce protection. To harness this promise, a more determined and imaginative approach to preventive medicine and public health is required. Provided government agencies can see the economic as well as the health benefits of developing such approaches, the benefits to mankind and human health could be immense. □

A.J. Beale is at Wellcome Biotechnology Ltd., Beckenham, Kent BR3 3BS, UK.

γ-ray sources

Does Geminga exist yet?

from Roger W. Romani and Virginia Trimble

THE peculiar γ-ray source in the constellation Gemini, termed Geminga, is once again puzzling astronomers. Originally found by the SAS-2 satellite¹ and later studied by γ-ray detectors both in space² and on Earth, this object presents strange features in every wavelength band. One of the strangest — that it cannot be detected at visible and radio wavelengths — is reflected in its name, which also means "does not exist" or "is not there" in Milanese dialect. New optical observations (refs 4–6 and G.F. Bignami *et al.*, in preparation) have provided a tentative identification but these make Geminga seem even more inexplicable: as G.F. Bignami (Milan) reported at a recent meeting*, the object is exceedingly faint and

may have a very large proper motion.

As the brightest of the 20 unidentified γ-ray sources in the COS-B catalogue³, Geminga is a natural subject of searches for corresponding sources in other energy ranges. The high count rate and large distance from the confusion at the galactic centre allowed the COS-B collaboration to obtain a position that is excellent by γ-ray standards. Images taken with the Einstein X-ray satellite's high resolution imager (HRI) and imaging proportional counter (IPC) led to the identification of Geminga with the bright source 1E0630+178 (ref. 7), supported, it then seemed, by the same 59–60-second, gradually lengthening pulse period in both X- and γ-ray data⁴. Buccheri *et al.*⁴ and others have doubted the periodicity, without necessarily disbelieving the identification, because there is unlikely to be an unusual

*NATO Advanced Study Institute on "High Energy Phenomena around Compact Stars" held at Cargèse, Corsica, 2–13 September, 1985.

X-ray source and a bright γ -ray source in one 0.4 square degrees of sky.

Searching at lower frequencies and with greater positional accuracies has met with some difficulty. Radio maps of the γ -ray error box (refs 10–12 and V. Boriakoff *et al.*, unpublished data) reveal more than a dozen 10–100 mJy sources, but there is nothing at the X-ray position and nothing particularly unusual anywhere.

At optical wavelengths, deep charge-coupled device exposures taken at the Canada–France–Hawaii telescope in January 1984 revealed a 21st magnitude image marginally within the Einstein error circle¹³. Dubbed the G candidate, it has been closely scrutinized by a number of groups. Discouragingly, optical and infrared photometry and spectroscopy (refs 4,5,14,15 and M.J. Lebofsky, unpublished data) indicate that the candidate probably is just a G-type star — a cool white dwarf 100–200 pc from us or, more likely, a slightly hotter main-sequence or subdwarf star several thousand parsecs away. Both are inconsistent with the Einstein X-ray colour temperature of about 10⁶ K (ref. 7) and the latter is also inconsistent with the very low X-ray absorption ($\leq 2 \times 10^{20}$ H cm⁻²), which indicates a distance less than 250 pc.

Moreover, a careful search for optical pulsations at a range of periods around those reported in X and γ -rays¹, has set 2–4 per cent limits on any variability of the G candidate. The sky, according to Lebofsky, is full of similar stars and the chances of this one having anything to do with Geminga are slim.

Meanwhile, back at Lick Observatory, Djorgovski and Kulkarni⁴ have searched deeper at the HRI position and found two fainter candidates: G' at 24.5 mag near the centre of the error circle and G'' at 25½–26 mag off to one side. The lack of any brighter optical counterpart has interesting implications for 1E0630+178. The ratio of X-ray to optical luminosity is about 1,000 if G' is the counterpart, 2,500 if G'', and $\leq 3,000$ if neither is — among identified Einstein sources, only the radio pulsars and low-mass X-ray binaries have such large ratios. The absence of a detectable radio signal argues against a radio pulsar and the faintness of G' would place a typical X-ray binary at the improbable distance of 200,000 pc, arguing against an X-ray binary. This makes 1E0630+178 unlike any other known X-ray source, whatever association it has with Geminga.

G' and/or G'' seem to be peculiar, in any case. Djorgovski and Kulkarni⁴ report that G' has a tentative proper motion of 0.6 seconds per year; an alternative explanation is that this reflects a larger motion of G' plus spillover of light between the images. In addition, the image that Bignami and his collaborators have obtained by summing the twelve exposures made in January 1984 shows a faint object that could be G''. But its position is very different from that found at Lick in

March 1985, implying a proper motion of 1.4 seconds per year for G'' and an upper limit of ≤ 0.2 seconds per year for G'.

Its rapid motion, if confirmed, must place the star quite close to us; for example, even a halo object moving at 200 km s⁻¹ would be no more than 30 pc away. What could it be? From the magnitudes, G' and G'' could be interpreted as brown dwarfs, not unlike the recently-discovered companion of vB8 (ref. 16). But such a star is most unlikely to be either the X-ray source or a chance superposition; if there were a single brown dwarf so close to us in every 10⁵ Einstein error circles ($r = 3.3''$), it would make up the entire local 'missing mass' and can therefore be excluded from searches of larger areas.

It is somewhat more likely that G' or G'' is the thermal emission from a neutron star. Because the optical effective temperature could be anywhere from the X-ray colour temperature (10⁶ K at the hot polar caps) to the X-ray effective temperature ($\sim 3 \times 10^5$ K), the implied distance is quite uncertain. Possible combinations are G'' with the lower temperature and a runaway velocity of about 200 km s⁻¹ at 20–30 pc, or G' with the higher temperature and atypical young star velocity of ≤ 50 km s⁻¹ at 80–100 pc.

Not even a white-dwarf binary companion^{17,18} could remain hidden at these distances, which leaves only binary¹⁹ or single²⁰ neutron stars as viable models. Either is possible in energetic terms, if the 60-second period is real and can be attributed to orbital motion or rotation in the two cases, although this then means γ rays must be grossly non-thermal. But both models imply soberingly short lifetimes, about 700 years, and the second may also

have difficulties in explaining the acceleration of particles to the energies needed to emit γ rays. The short lifetime would make sense if the 'guest star' of +437 gave birth to Geminga⁸ but the total absence of emission nebulosity in the vicinity⁹ argues against any recent local violence.

Clearly, one or two more deep images of the region, confirming or refuting the rapid motion of G'' and providing a bit of colour (temperature) information on the two candidates, will be critical for sorting out the confusion, in which Geminga, 1E0630+178 and G' or G'' could be one, two or three interesting objects. □

1. Fichtel, C.E. *et al.* *Astrophys. J.* **198**, 163 (1975).
2. Swanenburg, B.N. *et al.* *Astrophys. J.* **243**, L69 (1981).
3. Zyskin, Y.L. & Mukanov, D.B. *Sov. AJ Lett.* **9**, 117 (1983).
4. Djorgovski, S. & Kulkarni, S.R. *Astr. J.* (in the press).
5. Kulkarni, S.R. & Djorgovski, S. *Astr. J.* (in the press).
6. Vigroux, L., Paul, J., Delache, P., Bignami, G.F. & Caraveo, P.A. *18th ESLAB Symp.* (1985).
7. Bignami, G.F., Caraveo, P.A. & Lamb, R.C. *Astrophys. J.* **272**, L9 (1983).
8. Bignami, G.F., Caraveo, P.A. & Paul, J.A. *Nature* **310**, 464 (1984).
9. Bucheri, R., D'Amico, N., Hermsen, E. & Sacco, B. preprint.
10. Sieber, W. & Schlickeiser R. *Astr. Astrophys.* **113**, 314 (1982).
11. Spelstra, T.A. & Hermsen, W. *Astr. Astrophys.* **135**, 135 (1984).
12. Caraveo P.A. *et al.* *Adv. Space Res.* **3**, 77 (1984).
13. Caraveo, P.A., Bignami, G.F., Vigroux, L. & Paul, J.A. *Astrophys. J.* **276**, L45 (1984).
14. Sol, H. *et al.* *Astr. Astrophys.* **144**, 109 (1985).
15. Halpern, J.P., Grindlay, J.E. & Tytler, D. *Astrophys. J.* **296**, 190 (1985).
16. McCarthy, D.W., Probst, R. & Low, F. *Astrophys. J.* **290**, L9 (1985).
17. Bisnovatyu-Kogan, G.S. *Nature* **315**, 555 (1985).
18. Arons, J. preprint.
19. Nulsen, P.E.J. & Fabian A.C. *Nature* **312**, 48 (1984).
20. Katz, J. *Astrophys. Lett.* **24**, 183 (1985).

Roger W. Romani is at the California Institute of Technology, Pasadena, California 91125; Virginia Trimble is at the University of Maryland, Maryland 20742 and University of California, Irvine, California 92717, USA.

Plant sciences

Molecular view of pollen rejection

from Deborah Charlesworth

IN MANY species of flowering plants, a genetic self-incompatibility mechanism causes the rejection of pollen from the same plant. The genetic control is determined by one or more self-incompatibility (S) loci and it has been clear for several years that many of the mysteries surrounding the S locus will be resolved only when the molecular structure of the locus is known. A start in that direction is provided by J.B. Nasrallah and colleagues on page 263 of this issue¹.

Two types of self-incompatibility are known². In the Compositae and Cruciferae, and perhaps also in some other families^{3,4}, control of the pollen reaction is sporophytic — that is, the pollen type is determined by the genotype of the plant that has produced it — and there is usually, perhaps invariably, a single S locus. In gametophytic systems, which are known in several families and where the pollen

type is controlled by the alleles in the pollen grains, there is often one but may be two S loci, as in the grasses, or even more; *Beta vulgaris* has four⁵. With both types of system, there are very large numbers of alleles at the S loci, so that although the genetic data indicate that the S loci control the specificity of the reactions, which must be expressed both in pollen and in the stigma or style in order for self-incompatibility to result, there has been a recurring tendency to doubt whether the sequence information for all the S glycoproteins is really encoded at the S locus, and to suggest that some or all of it must exist elsewhere in the genome, and that the S locus somehow switches between specificities^{2,7}.

Nasrallah *et al.* already have some data relevant to this question. They have cloned DNA from the S₅ allele of *Brassica oleracea* and shown that the protein encoded by the S DNA is detected by an

antibody to the S_h glycoprotein produced in intact stigmas. Thus the S locus must contain the sequence that encodes the S_h glycoprotein, although it remains possible that other loci also contain S DNA sequences. A test of that possibility will be to determine whether all clones isolated from a given homozygous genotype contain the same DNA sequences, which would suggest that they are all derived from a single locus. Alternatively, the clones may reveal the presence of multiple self-incompatibility loci, even if they are very tightly linked. Nasrallah *et al.* have probed digests of the genomic DNA of homozygous lines with several S alleles with their S_h probe; although so far they have only used a single restriction enzyme, each allele has been shown to give a unique pattern, and the variation suggests multiple differences will be revealed by the use of further restriction enzymes. On the other hand, the results provide preliminary evidence suggesting that there is only a single locus; if there were many loci, one would expect to see a complex pattern of many bands, varying in intensity as well as mobility.

Another very important question that can now be studied is whether the S specificity is determined by the protein or the carbohydrate part of the molecule. The high number of S alleles in self-incompatible plant species makes it hard to imagine that the carbohydrate part alone could be responsible. Now that the DNA can be obtained, it will be easy to see how much the amino-acid sequences of the S proteins differ, and whether this variation is in the position and nature of attachment sites for the carbohydrates. Ultimately, it should be possible to look for regions concerned with the different functions of the S protein. At present, it is not even clear whether the three functions of specificity, and activity in the pollen and stigma or style are controlled by three separate, but tightly linked cistrons, as suggested by Lewis¹⁵ on the grounds that the three functions are independently alterable by mutation, or whether they could be under the control of a single locus with separate functional domains. Nor do we know whether the antigenic specificities used in detecting S proteins have anything to do with the regions that are important for the plants' recognition reactions.

A related puzzle is that apparently mutations of one S allele to another do not occur⁹. Except during another culture¹⁰, such changes have so far been detected only under conditions of inbreeding^{11,13}, thus if anything deepening the mystery. Since S loci are highly polymorphic, in all species where any relevant data exist, mutation must occur to balance chance loss of alleles. The rarity of S mutations might be because existing S alleles differ at multiple amino-acid sites. Assuming that only a subset of possible sequences of the S protein can function as self-incompatibility alleles, one would not be able to get

mutation from one S allele to another, but could get mutant types that lose the ability to be processed in pollen or style (as in Lewis's pollen-part and stylar-part mutations). Molecular studies should help to test these ideas, and should suggest why mutations are so rare.

Once the S gene is characterized in one species, it will be possible to probe for the S genes of related species, which will be of great practical significance. Indeed, it may be the best way of genotyping plants for the S locus, given the difficulty of genetic methods of testing and the uncertain success of techniques using the proteins, such as immunological methods¹⁵⁻¹⁷.

Eventually, this type of work should enable us to determine whether S loci in different families of plants are homologous. The S DNA isolated by Nasrallah *et al.* will be useful with other sporophytic systems, but one can hope that DNA from species with gametophytic systems will be isolated in the future. It may then be possible to find out whether S systems are homologous in all angiosperms¹⁸, or have

evolved more than once, as seems likely from the distribution of the different types of system¹⁹. □

1. Nasrallah, J. B., Kao, T.-H., Goldberg, M. L. & Nasrallah, M. E. *Nature* **318**, 263 (1985).
2. De Nettancourt, D. *Incompatibility in Angiosperms* (Springer Verlag, Berlin, 1977).
3. Koyama, Y., Shimano, N. & Kawasaki, T. *Theor. appl. Genet.* **58**, 149 (1980).
4. Lundqvist, A. *Heredity* **91**, 307 (1979).
5. Thompson, M. M. *Theor. Appl. Genet.* **54**, 113 (1979).
6. Larsen, K. *Heredity* **85**, 227 (1977).
7. Mulcahy, D. L. & Mulcahy, G. B. *Science* **220**, 1247 (1983).
8. Lewis, D. *Proc. 11th Int. Cong. Genetics* (ed. Geerts, S. J.) **3**, 656 (1963).
9. Lewis, D. *Heredity* **5**, 399 (1982).
10. Sree Ramulu, K. *Heredity* **49**, 319 (1982).
11. Van Gestel, A. G. J. & de Nettancourt, D. *Incompatibility Newsletter* **6**, 6 (1975).
12. Denward, T. *Heredity* **49**, 189 (1963).
13. Sree Ramulu, K. *Incompatibility Newsletter* **14**, 103 (1982).
14. Wright, S. *Genetics* **24**, 538 (1939).
15. Sedgley, M. *Euphytica* **23**, 543 (1974).
16. Raff, J. W., Knox, R. B. & Clarke, A. E. *Planta* **153**, 125 (1981).
17. Hinata, K. & Nishio, T. *Heredity* **41**, 93 (1978).
18. Whitehouse, H. K. *Ann. Bot. new ser.* **14**, 198 (1950).
19. Charlesworth, D. in *Evolution: Essays in honour of John Maynard Smith* (eds Slatkin, M., Harvey, P. H. & Greenwood, P. J.) **237** (1985).

Deborah Charlesworth is in the Department of Biology, University of Chicago, 1103 57th Street, Chicago, Illinois 60637, USA.

Petroleum geochemistry

Advance in kerogen analysis

from R. P. Philp

It is commonly accepted that kerogen is a key intermediate in the formation of crude oil and/or natural gas from the organic debris of algae, bacteria, higher plants and other sources. Kerogen (Greek = oil or wax forming) is best described as the insoluble organic residue that remains after the organic solvent-soluble material, carbonates and silicates have been removed from the source rock or shale. Kerogen itself is formed following a series of complex chemical and, probably, microbial reactions involving the organic debris of higher plants, animals, algae, bacteria, spores, fungi, phytoplankton and zooplankton that is deposited in aquatic and reducing environments which favour the maximum preservation of organic material. Information on the nature of the organic source material responsible for its formation, its structure, the type of fossil-fuel products it will yield and its relative level of maturity is extremely important for petroleum exploration purposes. The paper by S. R. Larter and J. T. Senftle on page 277 of this issue reports a significant advance in the use of microscale pyrolysis techniques combined with gas chromatography (py-GC) for obtaining such data.

Most of the data used in oil exploration are based on analysis of oils and the soluble fractions of rocks. The kerogen in rock can be thought of as being indigenous, whereas it is always possible that the soluble fraction has migrated through the rock. The bulk of kerogen in source rocks and shales is insoluble in virtually all organic solvents and thus more difficult or

impossible to analyse by conventional methods such as gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS).

Microscale pyrolysis combined with GC and/or GC-MS provides a very powerful technique for kerogen characterization because a great deal of the geochemical data locked into the insoluble structure of kerogen can be released. Subsequent analysis of the pyrolysis products by GC provides information on the source material, thermal history and type of liquid products their host rock can generate. Another major advantage over analysis of the soluble material from the same rock is that all contaminants are normally removed during isolation of the kerogen, before characterization by py-GC. A major significance of Larter and Senftle's work is that it is a first attempt to quantify py-GC data for application to geochemistry. Equally important is the recognition of eight separate modes of kerogen composition from a number of cross plots involving normal hydrocarbon, aromatic hydrocarbon and total hydrocarbon yields obtained from the characterization of the kerogens by py-GC.

The word pyrolysis has been used in the geochemical literature to describe many different types of experiments, which has led to some confusion, to say the least. This is not the place to attempt a detailed classification but one broad distinction that needs to be made is between hydrous and anhydrous pyrolysis experiments. The work of M. D. Lewan *et al.* (*Science*

203, 897; 1979) greatly stimulated the use of hydrous pyrolysis techniques for evaluating the oil-generating potential of the source rocks from a particular basin. In this approach the source rock is sealed in a high pressure bomb with an appropriate amount of water and heated for varying periods at different temperatures. Obviously, water is not added to anhydrous experiments, but because crushed source rocks are very rarely completely free of water, it is doubtful if such experiments are ever truly anhydrous unless special conditions are observed.

Because of the apparent lack of alkenes in the products, the hydrous technique was promoted as the only method that could truly simulate the generation of crude oils in the laboratory, but it is more correctly thought of as a thermal simulation experiment in a closed system. In this situation, alkenes are unstable and are either transformed to saturated forms or react with the organic matrix. Evidence is, however, now available to show that alkenes can be generated under certain conditions of hydrous pyrolysis and their generation can be eliminated under certain conditions of anhydrous pyrolysis.

The most useful applications of hydrous pyrolysis are to simulate the effects of natural maturation in the laboratory and to determine the petroleum potential of a particular stratigraphic sequence at different burial depths within a basin. With one exception, the main application of anhydrous pyrolysis in geochemistry has been for typing kerogens and their possible precursors to determine the nature of the products that would result from the additional maturation or burial of the kerogen. The exception is the widely used Rock Eval pyrolysis method, widely used in the petroleum industry for routine and rapid screening of samples as they are recovered during the drilling of a well. In this method a crushed rock sample is subjected to a temperature range from ambient to 550° and the products analysed using a flame-ionization detector. Two chromatographic peaks are observed, the first relating to material normally extracted by solvents, the second to the products of thermal degradation of the kerogen. The temperature at which the second peak maximizes can be related to the maturity of the sample, and ratios of the two peaks provide information on the petroleum potential of a source rock.

Larter and Senftle report an advance in the technique of anhydrous pyrolysis, which commonly involves the combination of the pyrolysis system with either GC or GC-MS and permits fingerprinting of kerogens on the basis of the distribution of their pyrolysis products. (Although alkenes are produced during the pyrolysis reaction, this should not be looked on as a detrimental feature of the technique because alkenes still represent structural features of the kerogens which, in the natural situation, would be hydrogenated to the

corresponding alkane.) Larter and Senftle now describe how the use of polymethylstyrene as an internal standard permits the accurate quantitation of pyrolysis yields. The advantage of this approach is illustrated where two quite different macerals produce pyrograms that are indistinguishable from a qualitative point of view but can be differentiated by quantifying the yields of pyrolysis products. The study also shows that cross plots of the $C_7 - C_{25}$ normal hydrocarbons versus the $C_6 - C_8$ aromatics permit the different types of kerogens to be clearly observed. A further level of differentiation is obtained by plotting $C_{10} - C_{30}$ normal hydrocarbon concentration versus the hydrogen index from the Rock Eval data (equivalent to H/C ratio), which in turn permits the differentiation of eight types of kerogen.

The past five years have seen tremend-

ous developments in the application of geochemistry to petroleum exploration in areas such as oil/oil and oil/source correlations, maturity, migration, source determination and basin modelling studies. Much of this has been based on solvent-extractable organic compounds. The next five years will see a similar expansion using of information from py-GC characterization of kerogens, together with partially insoluble material of high molecular weight, the asphaltenes. Microscale pyrolysis will be a key to eliminating confusion arising from the use of the soluble fraction; the quantitation described by Larter and Senftle will be valuable for the technique's continuing success. □

R. P. Philp is in the School of Geology and Geophysics, University of Oklahoma, Norman, Oklahoma 73019, USA.

Molecular evolution

The uniqueness of Archaeobacteria

from Roger A. Garrett

UNTIL recently, orders of unusual microorganisms that had adapted to a peculiar niche, or exhibited a biochemical oddity, were considered to be subdivisions of the prokaryotes. Superficially, this classification was tenable. But once genetic information, or nucleotide sequences, of the microorganisms were examined closely, some species were revealed to be as different from the other prokaryotes, or eubacteria, as they are from the eukaryotes. Consequently these organisms have been classified by Carl Woese and colleagues (*Science* 209, 457; 1980) as a third primary kingdom, the Archaeobacteria. Despite the evidence of their nucleotide sequences and several distinctive biochemical characteristics, this assignment has received a sceptical response from some quarters, but the participants of a workshop on the molecular biology of the archaeobacteria* should have been convinced by the plethora of unusual and unexpected characteristics reported.

The unique features of the nucleotide sequences have been demonstrated by Woese and colleagues for the 16S-like RNA molecule from the smaller of the two ribosomal subunits. They chose this molecule for at least two reasons: it occurs in every living organism and its functional role in protein biosynthesis has remained constant throughout evolution. Moreover, in the cytoplasmic ribosomes of eukaryotes, although its size increases substantially it retains the core structure common to the prokaryotic RNAs. Comparison of partial and later of whole RNA sequences produced coefficients for the degree of relatedness between organisms. It was these coefficients that first revealed

the deep divide amongst the prokaryotes.

This division quickly received support from biochemical studies. The membrane lipids of the archaeobacteria were shown to have ether-linked and branched aliphatic chains, whereas those of eubacteria contain ester-linked and straight aliphatic chains. Moreover, the DNA-dependent RNA polymerases from archaeobacteria yielded more complex subunit patterns than those of eubacteria.

The archaeobacteria consist of three main orders: the extreme halophiles, the methanogens that metabolize carbon dioxide to produce methane, and the sulphur-dependent extreme thermophiles that include the Thermoproteales (comprising the families Thermoproteaceae, Desulfurococcaceae, Thermofilaceae and Thermococcaceae) and the Sulfolobales that grow under very acidic conditions; only the species *Thermoplasma* does not fall into any of these orders but occupies an intermediate position. Each order has some special characteristics that reflect adaptation to a particular niche, as exemplified by the heat-stable RNA structure in the extreme thermophiles, but other characteristics are common to all archaeobacteria.

Among the archaeobacterial characteristics reported at the workshop were uniquely modified nucleotides in tRNAs, unique features of the secondary structures of each of the ribosomal RNAs, different ribosome shapes and sensitivities to antibiotics, DNA-dependent RNA-polymerase subunits that seem to be like those of eukaryotes, unique reverse gyrase that produce positive super-coils in closed circular DNA and nucleosome-like structures; there was also evidence of unusual modes of gene transfer.

*EMBO Workshop "Molecular Genetics of Archaeobacteria", Martinsried, West Germany, 23-25 June, 1985.

The instability of the genome of *Halo-bacterium halobium* and of closely related halophiles received special attention. Recent studies of its chromosomal organization and of various extra-chromosomal elements (W. Goebel, University of Würzburg and F. Pfeifer, University of California, San Francisco) shed some light on the structural basis of the instability. The main chromosomal fraction, FI DNA, is 68% G+C-rich and can be readily separated from the more A+T-rich FII DNA fraction. The latter is complex and contains a heterogeneous collection of covalently closed circular DNAs, including a large plasmid carrying many insertion elements, as well as various minor components. Also present in the FII DNA are non-circular 70 kilobase (kb) 'islands' that probably associate with the chromosomal FI DNA; these also contain inser-

tion elements and participate in the genetic variation. A phage, ϕ H, isolated from the halophiles, also exhibits a high degree of genetic variability. No conclusive explanation was offered for the functional significance of the lability of the *H. halobium* genome, although some genomic changes seem to be reversibly inducible by changes in external salt concentration.

Exciting developments were reported in the characterization of virus-host systems (W. Zillig, Max-Planck-Institut für Biochemie, Martinsried) that will prove valuable in future for investigating the molecular genetics of the archaeobacteria. They included the nucleotide sequence of the virus-like SSVI from *Sulfolobus solfataricus* that has a 16-kb genome, and the identification of four viruses from *Thermoproteus tenax*, that partially resemble eukaryotic viruses in their structural orga-

nization. The ϕ H phage and the plasmid pSL10 from *S. ambivalens* are especially suited to vector development; other potentially important phage and plasmid vector systems were described for the methanogens.

The regulation of gene expression also figured prominently, with emphasis on the more readily isolated rRNA and tRNA genes and the bacterio-opsin gene of *H. halobium*. The observation of multiple transcriptional starts for the single rRNA operon of *H. cutirubrum*, occurring at regularly repeated sequences that include the motif 5'-A-A-G-T-A-A-3', was unexpected (P. Dennis, University of British Columbia, Vancouver). Another putative promoter sequence 5'-G-A-A-N-T-T-T-C-A-3' was deduced from sequencing the *purE*, *proC*, *hisA* and *argG* genes of various methanogens expressed in *Escherichia coli* (J. Reeve, Ohio State University, Columbus). Termination sites are less easily found; although some potential signals resemble those of eubacteria, others do not — it was suggested that five consecutive thymidines might be sufficient for ending the methyl-CoM-reductase gene of *Methanococcus voltae* (A. Klein, Phillips University, Marburg).

The detection of introns among the extreme halophiles and in a thermoproteale was a surprise. A 105-bp intron has been found in the tRNA^{Trp} gene of *H. volcanii* and other halophiles, with a fairly well-conserved sequence (C. Daniels and W. Doolittle, Dalhousie University, Halifax). Moreover, a 622-bp intron containing an open reading frame has been located in the 23S rRNA gene of *Desulfurococcus mobilis* (J. Kjems and R. Garrett *Nature*, in the press) in a similar position to that of an intron in the corresponding gene of the lower eukaryote *Physarum polycephalum*. Although no introns have been reported for protein messenger RNAs, the new results, together with an earlier report of putative small introns in the tRNAs of the sulfolobales, suggest that the presence of introns within ribosomal and tRNAs constitutes another fundamental difference between archaeobacteria and eubacteria.

Various disputes arose concerning archaeobacterial origins and centred on the order in which the extreme halophiles, methanogens and the extreme thermophiles separated from one another and from eubacteria and eukaryotes. The proposal by J. Lake (University of California, Los Angeles) that the sulphur-dependent thermoproteales and sulfolobales should be considered a separate kingdom because a few of their biochemical properties differ markedly from those of the extreme halophiles and methanogens received an extremely negative reaction, as did his contention (Lake, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 3716; 1985) that the extreme halophiles should be grouped with eubacteria because their ribosome shapes are compara-

The loess region of China

DELEGATES from ten overseas countries recently attended the international symposium on loess research held in Xi'an, in the People's Republic of China (5–13 October, 1985), the first such meeting to be held in the classic Chinese loess region.

A 5-day field excursion before the meeting traversed several loess regions, distinguished by their geomorphological features, between Xi'an and Ansai, 400 km to the north. Our route crossed the broad Wei River flood plain which is mantled by loess and dissected by a few small gullies. Northwards, the hill country is dominated by long, loess-covered ridges termed liang. Limestone quarries along the road gave a unique opportunity to observe how the liangs are related to the underlying bedrock. Smaller, loess-covered hills, known as mao, separate this area from the flat-topped loess plateau, termed yuan. Here the loess is about 100–200 m thick and is extensively cultivated with wheat and maize, a little cotton and tobacco.

The most spectacular features of these yuans are the deep erosional gullies that dissect the plateau. In particular, near Luochuan, the gully is some 130-m deep and in the near-vertical cliffs a sequence of more than 15 palaeosols can be seen overlying the basal red clay. One site has been studied for about 30 years and the chronological sequence has been established primarily by palaeomagnetic measurements of a core nearby. These results imply that the 130-m deep section spans the last two magnetic epochs, the Brunhes and the Matuyama. Detailed magnetic stratigraphies reaching back into the Gauss normal epoch (Heller, F. & Liu, T-s *Nature* **300**, 431; 1982), indicate that loess deposition may have started as early as 2.66 Myr ago. This is similar to the 2.9 Myr date reported by G.J. Kukla (Lament-Doherty, Geological Observatory) for the beginning of

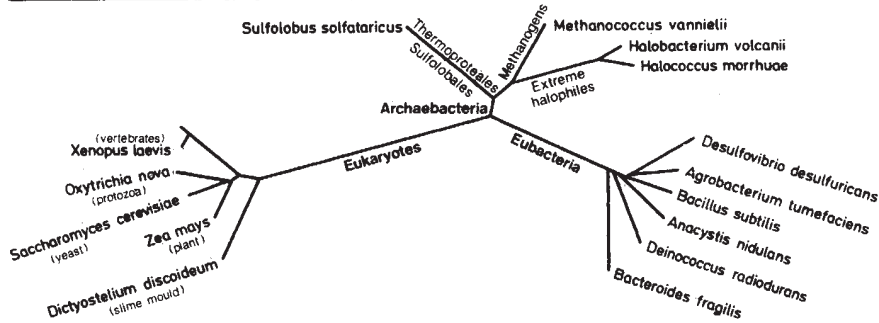
non-biogenic silica incorporation in a marine core off the Japanese coast. Similar and more continuous records are expected from the Qaidam Basin, closer to the source area of the loess, and it is tempting to correlate the palaeosols with the interglacial periods revealed by the deep-sea oxygen-isotope record.

Another feature of the symposium was the integration of geotechnical and chronological studies of loess. A large number of physical properties, such as the bulk dry-density and liquid limit, have been measured and related to the probability of landslides and collapse of loess when it is waterlogged. These properties are of interest as the area is bounded by major active faults and records document many major earthquakes in the past two thousand years.

A highlight of the field trip was a visit to an experimental conservation area, in Ansai County, Shaanxi Province, set up in 1980 by the Northwest Institute of Soil and Water Conservation of Academia Sinica. Erosion of the loess and subsequent landslides are major problems for agriculture, road maintenance and the safety of villages. Experiments designed to measure water run-off and removal of loess have been set up on the top of a hill overlooking the field station, and different types of shrubs and trees have been planted to try to stabilize the surface. We are also privileged to be the first foreigners to visit a village where the results of this research have been applied. Higher crop yields and more terracing have enabled the villagers to cover the upper slopes with trees and leguminous plants, which protect their livelihood.

Ann Wintle and Edward Derbyshire

Ann Wintle is at the Godwin Laboratory, University of Cambridge, Cambridge CB2 3RS, and Edward Derbyshire is Professor of Geography at the University of Leicester, Leicester LE1 7RH, UK.



Universal phylogenetic tree compiled from completed sequences of 16S-like RNAs. The lengths of the lines indicate sequence distances and are an approximate measure of evolutionary time. The figure was kindly provided by G. Olsen (Indiana University, Bloomington) and C. Woese (University of Illinois, Urbana).

ble and because it is unlikely that photosynthesis evolved in two separate events. In response, Woese (University of Illinois, Urbana) emphasized the importance of considering many biochemical characteristics when constructing phylogenetic trees and stressed that these problems should continue to be resolved at a molecular level. He then presented, for the first time, a universal phylogenetic tree (see the figure) based on recently completed 16S-like RNA sequences. The lines indicate the relative sequence distances between the archaeobacteria, eubacteria and eukaryotes but the root of the tree is not defined. Although he is convinced that 16S RNA is an excellent chronometer for evolution because of its universality and slow rate of change, Woese recommends that RNA sequence data should be supplemented by pro-

tein sequences including those of the subunits of the DNA-dependent RNA polymerases and of a protein not directly associated with the genetic apparatus, such as an ATPase.

There were optimistic messages for all participants. The biotechnologists were promised heat-stable enzymes from the extreme thermophiles and genetically engineered methanogens for improving industrial processes. The molecular geneticists were offered a wealth of novel and exciting problems. And the evolutionists were shown how to refute the age-old prejudice that first there were prokaryotes and then eukaryotes, and how to infer the nature of early forms of life on Earth. □

Roger A. Garrett is in the Division of Biostructural Chemistry, Department of Chemistry, Aarhus University, 8000 Aarhus C, Denmark.

Behavioural ecology

The limits to infanticide

from Paul H. Harvey and Anna Marie Lyles

THE true extent of infanticide among mammals is, it seems, only slowly becoming apparent. Over the years, new evolutionary explanations have been given to account for the limits to infanticide as currently perceived, but then a species crops up that breaks the rules. A new record of grisly proportions has been set by John Hoogland's observations on a wild population of black-tailed prairie dogs (*Cynomys ludovicianus*) in South Dakota¹. Offspring in more than half of the prairie-dog litters he observed are killed, usually by close kin.

Classical ethologists, such as Lorenz², argued that animals rarely kill others of their own species. Laboratory studies eventually showed that this was not true but, it was then claimed, laboratories are unnatural environments and infanticide is merely a maladaptive or pathological response to high population densities³. More recent field evidence has revealed that infanticide may be a predictable event in some natural populations. For example,

male lions (*Panthera leo*)⁴ and langurs (*Presbytis entellus*)⁵ entering a group regularly kill the young that were fathered by the males they replace.

Hrdy⁶ outlined a number of reasons why animals might kill young of their own species: the infants could represent a scarce resource, such as food (exploitation); the death of infants might increase the availability of other resources to its killer or relatives of its killer (competition); eliminating the dependent offspring of a possible future mate might increase the opportunities of mating access (sexual selection); parents might even be selected to kill particular offspring if investment in them was not likely to be worthwhile (parental manipulation); finally, some infanticide might not have adaptive significance and the eventual reproductive success of the killer could ultimately be reduced (social pathology). Competition and sexual selection account for most infanticide recorded among mammals⁷.

The main message of the most recent

News and Views article on infanticide⁸ was that, other things being equal, individuals are more likely to be selected to kill unrelated than related young, because the latter may be partially protected by kin selection. Indeed, it has been considered that, among mammals, close relatives rarely commit infanticide. In a book published last year, the editors wrote "For most mammals, however, with the exception of humans where mothers in certain circumstances may opt to abandon offspring, infants tend to be killed by unrelated individuals"⁷. Thus, although lion and langur males kill the young of their displaced rivals, thereby bringing the females back into oestrus more rapidly, the related females in a group do not kill each other's offspring, nor do males kill the young that they or their relatives are likely to have sired. Similarly, Sherman states that among Belding's ground squirrels (*Spermophilus beldingi*), "individuals never killed young of relatives (that is, cousins or closer kin)" even though "at least eight per cent of all young born were preyed on by conspecifics"⁹.

Hoogland's results are a stark contrast. Out of the 73 black-tailed prairie dog litters that he observed, 51 per cent lost either some (13 per cent) or all (38 per cent) of the offspring. Most of these were killed by lactating females, 80 per cent of which were close relatives — mother, daughter, sister, aunt, niece or cousin of the victims' mothers.

Why might natural selection favour killing the offspring of a relative? Hoogland suggests that infanticidal females, which he terms 'marauders', acquire valuable food by cannibalizing the young they kill (exploitation as defined by Hrdy⁶), which helps them with the successful weaning of their own litters. In support of this idea, Hoogland shows that marauders are in better condition than non-marauders when their young are weaned, and they wean more and heavier offspring. But there are also costs: prairie dog females live in burrows alone with their young and when a lactating female is out marauding, her offspring are left undefended. Three

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

A prairie dog (photography courtesy of the Zoological Society of London).

females lost their own young when committing infanticide elsewhere and two more lost theirs while visiting burrows where infanticide had recently occurred. Given these costs, infanticide is most likely to pay dividends if it involves nearby burrows. The problem is that nearby burrows are usually occupied by close relatives. Prairie dogs group into coterries, consisting of female kin with an unrelated adult male. All members of the coterie defend their territory against invasion from outsiders. Attacks on distant burrows occupied by non-relatives therefore entail greater risk in both time (absence from own burrows) and danger, since a foreign coterie's defences have to be breached, as well as those of an individual burrow. Possibly, the extra benefits in genetic terms may not be worth the extra costs. □

1. Hoogland, J.L. *Science* **230**, 1037 (1985).
2. Lorenz, K. *On Aggression* (Harcourt, Brace & World, 1966).
3. Calhoun, J.B. *Sci. Am.* **206**, 139 (1962).
4. Packer, C.R. & Pusey, A.E. in *Infanticide* (eds Hausfater, G. & Hrdy, S.B.) 31 (Aldine, 1984).
5. Hrdy, S.B. *Folia Primat.* **22**, 19 (1974).
6. Hrdy, S.B. *Ethol. Sociobiol.* **1**, 13 (1979).
7. Hausfater, G. & Hrdy, S.B. (eds) *Infanticide* (Aldine, 1984).
8. Jones, J.S. *Nature News and Views* **299**, 202 (1982).
9. Sherman, P. in *Natural Selection and Social Behavior* (eds Alexander, R.D. & Tinkle, D.W.) 311 (Chiron, 1981).

Paul Harvey is in the Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK; Anna Marie Lyles is in the Department of Biology, Princeton University, Princeton, New Jersey 08544, USA.

Charles F. Richter (1900–1985)

CHARLES Richter, co-developer of the earthquake magnitude scale which bears his name, died on 30 September, 1985. He lived in California most of his life and that is where he developed his fascination with earthquakes during the embryo stages of the new science of seismology. In 1927, at Robert A. Millikan's request, he accepted a position as a physicist at the newly established Seismological Laboratory which was later to become part of the California Institute of Technology. During the 1930s he collaborated with Beno Gutenberg on a series of seminal publications entitled *On Seismic Waves* published between 1934 and 1939 in *Beiträge zur Geophysik*. These studies laid the ground work for modern observational seismology and resulted in the first detailed models of the mantle and core.

This collaboration also led to various earthquake magnitude scales and to the first complete compilation of earthquakes, published as *The Seismicity of the Earth in 1954*. Richter's book *Elementary Seismology*, which appeared in 1958, is an encyclopedia of earthquake information and is still widely used. Don Anderson

Neuroanatomy

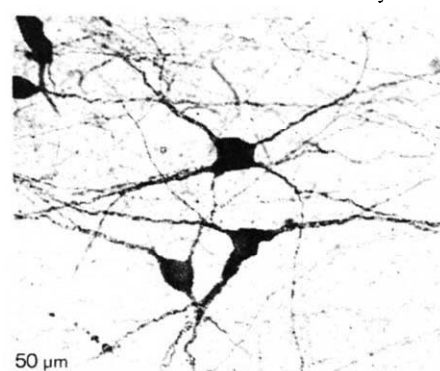
Antibodies to acetylcholine at last

from Felix Eckenstein

IN a recent publication, M. Geffard and colleagues describe the production of antibodies to the neurotransmitter acetylcholine (ACh) and the successful use of these antibodies for the immunohistochemical localization of ACh¹. This is a remarkable breakthrough in the effort to identify cholinergic structures in the nervous system; the more so as it has been held for a long time that ACh cannot be fixed in tissue, so that the molecule cannot be localized *in situ*. Geffard *et al.* have overcome this problem by using an ingenious way to modify ACh chemically within the tissue to a form that can be fixed and localized by antibodies prepared against ACh that has been modified in a similar way *in vitro*.

Acetylcholine was one of the first neurotransmitters to be described and the vertebrate neuromuscular junction, the classical example of a cholinergic synapse, is probably the system where the physiology of synaptic transmission has been studied in most detail. But our understanding of the systems of neurones in the central nervous system that use ACh as a transmitter is much more limited. The work of Geffard *et al.* adds an important new method to those already used for the anatomical identification of neurones that use ACh as their transmitter.

Up to now, the methods for the anatomical identification of cholinergic neurones have included the histochemical localization of acetylcholinesterase (AChE), the enzyme involved in the hydrolysis of ACh^{2,3}; immunohistochemical localization of the enzyme for synthesizing ACh, choline acetyltransferase (ChAT)^{4,5}; or dry autoradiography to localize sodium-dependent high-affinity choline uptake (HACU)⁶. It has become clear, however, that AChE is also present in a variety of non-cholinergic neurones^{8,10}, which limits its usefulness as a marker. Localization of HACU is technically diffi-



Photomicrograph of ChAT immunoreactivity in 30–40 μm diameter multipolar neurones in the basal forebrain of the rat. In this area, neurones of similar morphology have been labelled by Geffard *et al.*¹ using their antibodies to ACh.

cult and is also not entirely specific because non-cholinergic cells, such as rabbit photoreceptors, display HACU¹¹. So far, ChAT has been the only reliable marker for cholinergic neurones, although controversy about the specificity of antibodies to ChAT has plagued the field for a long time¹². Now that pure ChAT and monoclonal antibodies against the enzyme have become available^{1,6}, ChAT-immunohistochemistry is very useful for identification of cholinergic neurones.

In addition to confirming results obtained with antibodies to ChAT, the immunohistochemical localization of ACh offers other advantages. First, due to the limited cross-reactivity of ChAT-antibodies from different species, cholinergic neurones could be identified by this method only in vertebrates, whereas antibodies to ACh should have broader cross-reactivity. Second, the method for the preparation of antibodies to ACh described by Geffard *et al.* is simple enough for others to follow easily, whereas the preparation of antibodies to ChAT requires cumbersome purification of the enzyme.

One potential problem of the method lies in the way ACh has to be modified to allow its fixation. This step involves the substitution of the acetyl group of ACh with another group that is subsequently fixed in the tissue. It seems possible that this method will not only identify cholinergic neurones but also structures containing other choline metabolites. The rabbit photoreceptor, because of its high choline metabolism, should be a good model for testing this reservation. On the other hand, it is encouraging that the staining pattern obtained by Geffard *et al.* closely resembles that seen with ChAT-immunohistochemistry (see figure). This novel method is a more than welcome addition to the field, and one that is likely to add considerably to our understanding of the organization of cholinergic systems. □

1. Geffard, M., McRae-Degeurce, A. & Souan, M.L. *Science* **229**, 77 (1985).
2. Koelle, G.B. & Friedenwald, J.S. *Proc. Soc. exp. Biol. Med.* **70**, 617 (1949).
3. Shute, C.C.D. & Lewis, P.R. *Nature* **199**, 1160 (1963).
4. Eckenstein, F. & Thoenen, H. *EMBO J.* **1**, 363 (1982).
5. Houser, C.R., Crawford, G.D., Barber, R.P., Salvaterra, P.M. & Vaughn, J.E. *Brain Res.* **266**, 97 (1983).
6. Levey, A.L., Armstrong, D.M., Atweh, S.F., Terry, R.D. & Wainer, B.H. *J. Neurosci.* **3**, 1 (1983).
7. Baughman, R.W. & Bader, C.R. *Brain Res.* **138**, 469 (1977).
8. Butcher, L.L., Talbot, K. & Bilezikian, L. *J. Neural Transm.* **37**, 127 (1975).
9. Eckenstein, F. & Sofroniew, M.V. *J. Neurosci.* **3**, 2286 (1983).
10. Levey, A.L., Wainer, B.H., Mufson, E.J. & Mesulam, M.M. *Neuroscience* **9**, 9 (1983).
11. Masland, R.H. & Mills, J.W. *J. cell Biol.* **83**, 159 (1979).
12. Rossier, J. *Int. Rev. Neurobiol.* **20**, 284 (1977).

Felix Eckenstein is in the Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA.

Sequence curiosity in v-myc oncogene

SIR—The DNA sequences of three avian acute transforming retroviruses that contain the v-myc oncogene^{1,3} as well as the sequence of the chicken proto-myc gene⁴ have recently been reported. We have now noticed that there is a codon where as a result of adjacent mutations, none of the three viral sequences codes for the amino acid residue that is coded for by the cellular proto-myc gene. This is position 61 numbered from the first ATG in chicken exon 2 (Fig. 1). Position 61 and its neighbours are conserved among proto-myc genes from chicken⁴, human⁵, mouse⁶, and fish (R. Van Beneden, D. Watson and T. S. P., unpublished data).

Due to the relatively low number of amino acid differences between the viral and chicken myc sequences, it is rather unlikely that this has occurred by chance alone. Counting from the first ATG in exon 2, the chicken proto-myc locus codes for 416 amino acid residues. The number of amino acid substitutions between viral and chicken proto-myc is 7, 27, and 2 for MC29¹, MH2² and OK10³, respectively.

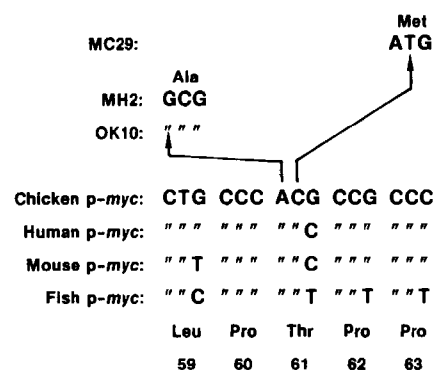


Fig. 1. Sequences of a segment of the myc gene from avian retroviruses and animal cells. The references for the sequences are given in the text. Except where noted, the viral sequences are identical to the chicken p-myc sequence for the segment shown. The codons are numbered from the first initiation codon in the second exon of chicken.

We calculate that there would be only one chance in 458 that all three viral sequences contain an amino acid substitution at a common position if the substitutions occurred randomly. We suggest as a possible explanation of this observation that substitutions at position 61 might lead to an increase in the oncogenic potential of the virus and thus would give the transformed cells a growth advantage.

We are currently evaluating this hypothesis by altering viral v-myc and animal proto-myc genes by site specific mutagenesis to determine if substitutions at position 61 indeed modulate oncogenic potential.

The occurrence of substitutions at a common location in the viral myc genes may be analogous to the findings in many

laboratories that codon 12 of a *ras* gene is altered in viruses that contain *ras* sequences and in tumour DNAs that are capable of transforming NIH 3T3 cells (see ref. 7 for review). While each of the three viruses that contain the *ras* gene differ from the normal sequence by a G to A transition in the first position of codon 12, the mutations in transforming tumor DNAs occur in either the first or second position. For example, the first position of codon 12 of the c-Ha-ras¹ allele of rat is altered in Harvey murine sarcoma virus and Rasheed rat sarcoma virus while the second position in this codon is altered in rat mammary carcinomas induced by nitrosomethylurea. Similarly, the human c-Ki-ras2 allele was found to be altered in the first position in a human lung carcinoma cell line and in the second position in a human colon carcinoma cell line.

TAKIS S. PAPAS

JAMES A. LAUTENBERGER

Laboratory of Molecular
Oncology, National Cancer Institute,
Frederick Cancer Research Facility,
Frederick, Maryland 21701, USA

1. Reddy, E. P. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **80**, 2500-2504 (1983).
2. Kan, N. C., Flordellis, C. S., Mark, G. E., Duesberg, P. H. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3000-3004 (1984).
3. Hayflick, J. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **82**, 2718-2722 (1985).
4. Watson, D. K., Reddy, E. P., Duesberg, P. H. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2146-2150 (1983).
5. Watson, D. K., Psallidopoulos, M. C., Samuel, K. P., Dalla-Favera, R. & Papas, T. S. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3642-3645 (1983).
6. Bernard, O., Cory, S., Gerondakis, S., Webb, E. & Adams, J. M. *EMBO J.* **2**, 2375-2383 (1983).
7. Varmus, H. E. *A. Rev. Genet.* **18**, 553-612 (1984).

Interferon and micRNA in cellular defence

SIR—Recently Coleman *et al.*¹ in a letter to *Nature*, proposed an original artificial micRNA (antisense RNA) system to prevent bacteriophage infection in *Escherichia coli*. A micRNA system has already been successfully used in mammalian cells² and micRNAs have been demonstrated to constitute normal prokaryotic mechanisms of regulation of gene expression at the translation level^{3,4}. The frequent finding in viral genomes of sequences transduced from the cellular genome makes attractive the hypothesis that the endogenous anti-sense transcription active in genetic regulation may also operate in some viral infections. We should like to suggest a link between micRNA and the interferon system of defence against viral infections.

Interferon induction requires virus penetration; either single or double stranded viral RNA constitutes the critical factor in triggering the induction process. The activation of the cellular antiviral state by interferons involves complex mechanisms, as yet poorly understood, but it is now well established that a crucial step in the activation mechanisms is the

presence of double-stranded RNA (dsRNA)⁵. dsRNA acts both as an affinity reagent and an activator for an enzymatic activity designated (2'-5') A polymerase which in turn activates an endonuclease, resulting in preferential cleavage of viral mRNA⁵.

Thus dsRNA is deeply involved both in the interferon induction and in the cellular metabolic pathways activated by interferon. This is a key point for our suggestion, that, upon virus infection and exposure to viral nucleic acids, the cell synthesizes micRNAs that are not only able to repress the translation of viral gene transcripts by binding to them but also thereby to constitute some of the dsRNAs that are involved both in interferon induction and in the cellular metabolic pathways activated by interferon.

Hybrids of a viral mRNA and an anti-mRNA in a partially double-stranded structure may also be preferentially cleaved by cellular enzymes which would account for the still unexplained discrimination between cleavage of cellular and viral mRNAs in interferon treated cells.

SERGIO GIUNTA

GIUSEPPE GROPPA*

Unit of Experimental Genetics and

*Section of Virology,
Clinical Laboratory,
INRCA, Ancona,
Italy

1. Coleman, J., Hirashima, A., Inokuchi, Y., Green, P. J. & Inouye, N. *Nature* **315**, 601-603 (1985).
2. Izant, J. C. & Weintraub, H. *Cell* **36**, 1007-1015 (1984).
3. Mizuno, T., Chou, M. Y., Inouye, M. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1966-1970 (1984).
4. Pestka, S., Daugherty, B. L., Jung, V., Hotta, K. & Pestka, R. K. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7525-7528 (1984).
5. Nilsen, T. W. & Baglioni, C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2600-2604 (1979).

Non-repellent bird droppings

SIR—I would like to propose an alternative explanation for the observation reported by L. Roodyn (*Nature* **317**, 581) that after rain, bird droppings on the roof of his car were surrounded by a dry zone, while large drops of water were scattered about the rest of the surface.

He suggested a water repellent substance in the droppings. I suggest just the opposite.

Droppings contain a detergent (perhaps bile salts), so rain falling on the droppings spreads over the surrounding roof in a thin film, which evaporates rapidly. Meanwhile, rain falling on other parts of the water-repellent roof (perhaps treated with silicone wax) forms rounded droplets with low surface area in relation to volume, and therefore takes longer to evaporate. Thus the water-repellent area is the wet one, not the dry one.

G. A. W. ROOK

Department of Microbiology,
Middlesex Hospital Medical School,
Riding House Street,
London W1P 7PN, UK

Catastrophism is still viable

SIR—It is true, as Weissman states¹, that Innanen *et al.* proposed that the Sun's galactic motion might correlate with geophysical periods. Similar claims have been made over the years for ice ages, mass extinctions, the Earth's magnetic field reversal frequency, the extrusion of carbonates in Canada and so on. Indeed Holmes some fifty years ago, without the benefit of radiometric dating or the plate tectonic revolution, pointed out that there were 30 Myr and 250 Myr cycles in the geological record. These long-standing claims, largely neglected and only now being rediscovered, were an important element in our conception of the galactic theory of terrestrial catastrophism. But what was missing from all the papers before 1978 was a *mechanism*. And although it seems to elude Weissman, a new idea has emerged, namely that the well known periodicities in the terrestrial record may arise because of the dominating influence of giant comets on the Earth's evolution and of the molecular clouds in the Galaxy in turn on their intermittent influx (see ref. 2 for a review, including references therein). Giant comets in particular take precedence because they contain most of the incident mass; the prolonged effects of the dust input from giant comets may therefore compete with the prompt effects expected from bombardments by their larger debris.

There have been claims recently of course that the mechanism does not work on the grounds that the Sun, unlike other stars of its age, must remain so close to the galactic plane that it cannot yield any significant modulation of the comet flux^{3,4}. However, such claims neglect the fundamentally stochastic nature of the galactic interaction, affecting the Sun's orbit as well as the comet cloud. Thus the current solar orbit is anomalously flat, tending to depress the 30 Myr component, but it experiences large gravitational deflections including a probable memory-erasing disturbance during the very recent encounter with Gould's Belt⁵. Terrestrial cyclicalities can only be avoided therefore if the Sun avoids molecular clouds, an eventuality that now appears to be hardly realistic.

By the same token it is misleading to favour stochasticity⁶ at the expense of cyclicity. It has been argued⁷ for example that the occurrence of large sea regressions immediately preceding the Permian and end-Cretaceous extinctions is too large a coincidence to swallow on the impact hypothesis. This comment is only applicable to the stray meteorite hypothesis⁸ however and would not apply to the galactic theory — we have ourselves pointed out that the two great mass extinctions were each preceded by the onset of a strong mixed magnetic interval. Thus the meteorite hypothesis predicts no cyclicalities

and, like Nemesis, seems to owe its erstwhile popularity more to Californian hype⁹ than the real astronomical environment.

Whilst it seems therefore that Weissman's quixotic comment has little bearing on the status of the galactic theory of terrestrial catastrophism, the question of how the Earth responds to the huge astronomical perturbations that are now expected remains one of formidable complexity, hardly yet tackled in a quantitative fashion, and to that extent one might agree that catastrophism is as yet unexplained.

S.V.M. CLUBE

Department of Astrophysics,
South Parks Road,
Oxford OX1 3RQ, UK

W.M. NAPIER

Royal Observatory,
Blackford Hill,
Edinburgh EH9 3HJ, UK

1. Weissman, P.R. *Nature* **316**, 572 (1985).
2. Clube, S.V.M. & Napier, W.M. in *The Galaxy and Solar System* (Tucson Symposium) University of Arizona Press, in the press.
3. Thaddeus, P. & Chanan, G.A. *Nature* **314**, 73–74 (1985).
4. Bahcall, J.N. & Bahcall, S. *Nature* **316**, 706–708 (1985).
5. Hoffman, A. *Nature* **315**, 659–661 (1985).
6. Van Valen, L.M. *Nature* **316**, 396 (1985).
7. Alvarez, L.W., Alvarez, W., Asaro, F. & Michel, H.W. *Science* **208**, 1095–1105 (1980).
8. Maddox, J. *Nature* **308**, 685 (1984).

Tenuous evidence for the luminous mouthed shark

SIR—The capture of a second specimen of the 'Megamouth' shark *Megachasma pelagios*¹ has prompted further discussion of its feeding mechanism by J. Diamond in a *News and Views* article². The published description of the first specimen³ noted that the lining of the mouth had a silvery appearance with pore-like structures and reported that "At the time [24 hours after its capture] it was speculated that these might be luminescent organs, but we have no evidence for this". These initial speculations were given more prominence in an earlier popular article⁴. The account of the second specimen describes the upper jaw and palate as "remarkably iridescent"⁵ and the previous speculations have been revived and developed by Diamond, who makes a number of assumptions in support of the premise that Megamouth has a luminous mouth into which the prey (euphausiid shrimps, copepods and jellyfish) are attracted. Beguiling though such a hypothesis may be, its factual basis is too tenuous for it to be allowed to go unchallenged.

The two specimens have been captured at epipelagic depths (< 38m and 165m) yet their "eclectic combination of habitus characters"⁶ suggest they are relatively weak swimmers. Diamond infers from this that they live "well below the rich plankton zone" and cannot support themselves by direct filter-feeding, unlike the whale shark and basking shark. He infers further

that because the prey is itself bioluminescent it would be attracted to an illuminated mouth. There are few grounds for either inference. The nighttime biomass peak of the micronekton in general and Megamouth's euphausiid diet in particular, lies in the upper few hundred metres, rather than right at the surface. Nor is there any direct evidence to suggest that euphausiids are attracted to a bioluminescent source. There is really no basis for speculation that the mouth is bioluminescent other than its unusual iridescence. Even so, although some luminous organs are silvery it does not follow that all silvery tissues are luminous. It could equally (perhaps more plausibly?) be argued that a reflective lining to the upper part of the mouth might make it less conspicuous than a wholly dark maw, and therefore more efficient in any daytime filter-feeding.

Like Diamond, I do not know of any filter-feeder that uses light to attract plankton (although it was once suggested as a function for the luminescence of the piddock, *Pholas*⁷). The answer to the question he poses of why filter-feeders do not attract prey into their mouths is probably a function of the difficulty of generating a non-specific signal of sufficient potency and range to supply the dietary needs. It is more likely that the problem of Megamouth's feeding methods will be solved by better understanding of both its sensory abilities and its jaw and filtration mechanics. The extraordinary nasal capsules, and the suggestion that Megamouth is more closely related to the whale shark than originally suspected⁸, may both be relevant in this respect.

The analogy drawn by Diamond, between the fishy example of Megamouth's proposed pelagic moth-trap and the feathered one of the frogmouth's nocturnal fly-paper, is certainly entertaining but probably more fabulous than factual.

PETER J. HERRING

Institute of Oceanographic Sciences,
Brook Road,
Wormley,
Surrey GU8 5UB, UK

1. Lavenberg, R.J. & Siegel, J.A. *Terra* **23**, 29 (1985).
2. Diamond, J. M. *Nature* **316**, 679 (1985).
3. Taylor, L.R., Compagno, L.J.V. & Struhsaker, P.J. *Proc. Cal. Acad. Sci.* **43**, 87 (1983).
4. Taylor, L.R. *Oceans* **10**, 46 (1977).
5. Damant, G.C.C. *Proc. Isle of Wight nat. Hist. Soc.* **4**, 155 (1951).
6. Maisey, J.G. *Copeia* (No. 1) 228 (1985).

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where the *Matters Arising* sections remains appropriate).

Down to the roots

William Coleman

The Background of Ecology: Concept and Theory.

By Robert P. McIntosh.

Cambridge University Press: 1985. Pp.383. £30, \$39.50.

ECOLOGY began in confusion and then prospered in confusion. At the outset an interest of very different enthusiasts, ecology has retained to a singular degree a seemingly irreducible diversity of subject matter. There has been much talk of theory, and the hope that one or another proposed theoretical programme would at long last provide the foundation for effective unification of the ecologist's concerns. That happy moment has not yet arrived. Each ecologist, it appears, is fated to seek his own, often highly personal view of the science, and not infrequently history has been used as a guide in that quest.

This situation is not unique. From classical antiquity to the nineteenth century, medicine, riven by conflicting theories of disease, turned to historical narrative and analysis in order to seek doctrinal coherence for purposes of further investigation and, above all, to afford the beginning student a feeling for the principal concepts of medicine and a sense of the structure of the profession. Chemistry followed a similar course. Prior to the revolution carried out by Guyton de Morveau, Lavoisier and Dalton, from which resulted a wide-reaching theoretical framework based on the regularities of combining proportions and a transformation of chemical language, the chemist often used a historical account of chemical investigations to give shape to his discipline and to impart to newcomers a sense of its accomplishments and problems. History was thus an integral part of medicine and chemistry, not an idle ancillary activity.

It appears that Robert P. McIntosh is following a similar course. *The Background of Ecology* is divided into eight chapters, arranged thematically. All revolve about the author's main concern, the rise of "self-conscious", that is, empirically and theoretically self-aware ecology, and each tends to offer in itself a complete chronological account. This procedure entails occasional but not objectionable repetition. There is, however, another and more serious drawback to McIntosh's approach. The structure of the book dictates an episodic view of the development of ecology. The topics covered — the emergence of early ecology from natural history, dynamic/community ecology, quantification and population ecology, ecosystems and systems ecology, the awesome expectations of theoretical ecology, and ecology as popularly understood (that is, conservation and the environmental movement) — appear as if on parade,

each in multi-coloured costume and announced by contentious advertising. While McIntosh is a spectator of this parade, indeed, a very attentive and extraordinarily well-informed observer, he only occasionally views the proceedings with a sharply critical eye and even more rarely seeks to relate to his readers the meaning of the great display.

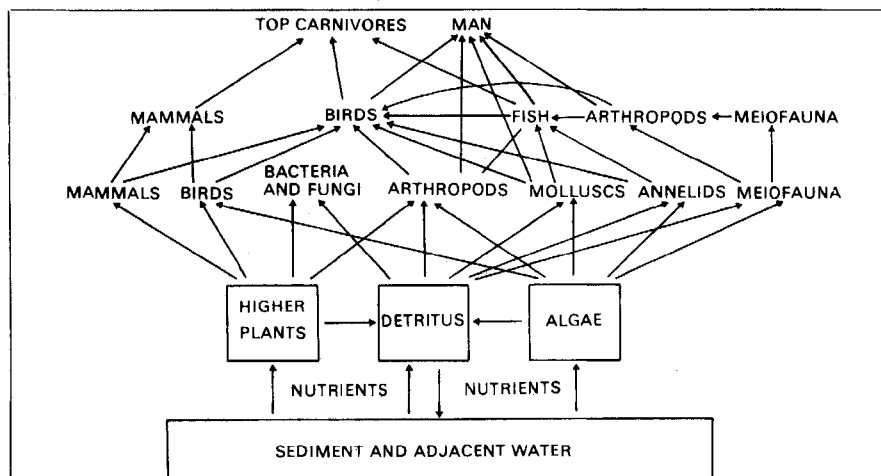
The Background of Ecology offers, in fact, a remarkable tally-sheet or taxonomy of who thought what and when, and who rose in opposition; in this lies one of its many virtues. It gives, however, little indication of why such opinions were entertained or rejected; what is largely missing from the book is a sustained analysis, an interpretation of ecology's history. Lacking this, we can conclude only that ecology has an old and respectable background and that, in more recent years, the science has pursued countless disparate objectives, all the while never being able to settle on a set of widely acceptable goals.

Of course, ecological ambitions have always been large and there is dubious merit in chiding an observer of the development of a science for not accomplishing what few of its practitioners themselves have accomplished. To early ecologists, as to their successors, it was relatively easy to state the needs of the science. Kerner von Marilaun, for example, announced in 1863 that the distribution of plants "follows immutable laws. Every plant has its place, its time, its function and its meaning". One's task followed directly therefrom: discover these laws. Nothing less would do for animals and, ultimately, for

the combined biotic world and for its relationship to the non-living world. Self-conscious ecology largely began with ambitions such as those expressed by Kerner. Botanists, exploring the spatial distribution and physiological adaptations of plants, especially the former, laid foundations for the science and created its first large-scale generalization, the notion of succession. Limnologists and marine scientists also stressed the idea of community, noting not only distinctive assemblages of organisms but emphasizing more than others the myriad interactions that bind these organisms together.

Until the 1920s, this was the primary fare of the ecologist. New possibilities then emerged. Under the protection of Raymond Pearl, and developed by Lotka, Volterra, Gause and others, the logistic curve and then two-species interactions swept animal ecology. The population now rivalled the species as the ecologist's principal interest and gradually the rich new domain of theoretical population ecology arose. Population ecologists turned to experiment and to mathematical refinement, but faced the charge that their premises represented serious oversimplification and their empirical studies ignored the complexity to be found in the field.

The 1930s announced the ecosystem concept, but that idea came into its own only after the Second World War. Joined by systems theory and enthusiastically supported by the United States Atomic Energy Commission and other agencies, ecosystem ecology became the favoured child of the post-war years. Its promise of efficiency, holistic thinking and ready applicability added to its attractions. In an unparalleled crescendo of organization and expenditure, ecosystem ecology emerged as the keystone of the International Biological Programme, ecology's debut in Big Science. McIntosh's account of these developments is both comprehensive and droll; he has a keen eye for excesses as well as for science and scientists.



An idea of ecological complexity — a simplified food web on a saltmarsh. (From *Saltmarsh Ecology* by S.P. Long & C.F. Mason; Blackie/Chapman & Hall, 1983.)

One of the fundamental problems in writing the history of ecology is the fact, itself not at all unexpected, that few new trends or interpretations displaced previous views and practices. The science persists and advances in a constantly unsettled condition. There has been and remains great dispute over the basic unit or units of ecological discourse — individuals, species, populations, communities, ecosystems or still larger entities; there is disagreement over the existence, character or relative importance of various ecological generalizations — succession, biogeochemical cycling, competitive exclusion; there is now available a host of research methods, undue allegiance to any one which might, as is always the case in the sciences, commit the investigator to one interpretive strategy and exclude other, perhaps promising possibilities. As noted, McIntosh has not elected to develop his history along these thematic lines.

One should attempt to do so, however, for what is needed today, in addition to detailed chronological accounts such as that offered here, is equally detailed analysis of several questions in the history of ecology. We need to know, for example, how and why animal ecology followed a course different from that of plant ecology; why population phenomena rose to the fore during the 1920s and 1930s; what role, if any, ecology played in the evolutionary modern synthesis; what have been the advantages of large-scale scientific research; and what have been the factors behind the shifting emphasis upon cooperative and competitive ecological models. We still need, despite the flood of literature seemingly directed to the topic, a clear view of the relationship of ecology as an aspiring natural science and the environmental movement as a political, social and moral campaign. The historian's obligation is to address such matters and not to rest content with a chronicle of events.

The Background of Ecology offers excellent preparation for such study. The author omits very little in the Anglo-American tradition — Continental ecology was not part of McIntosh's brief — and provides an up-to-date and virtually exhaustive bibliography of relevant primary and secondary materials. Moreover, his classification of doctrinal tendencies and portrayal of leading controversies provides a valuable guide to a vast and still largely uncharted sea. Regrettably, only within certain of its sections is McIntosh's volume a work of incisive interpretation, but it is throughout a reference work of distinction and one which is sure to be of interest to ecologists as well as to historians. □

William Coleman is Dickson-Bascom Professor in the Humanities and Professor of History of Science and History of Medicine at the University of Wisconsin, Madison, Wisconsin 53706, USA.

Private life of radiochemistry

Alwyn M'Kay

George de Hevesy: Life and Work. By Hilde Levi. *Rhodos International, Copenhagen/Adam Hilger: 1985. Pp.147. Dkr.120, £15, \$25.*

GEORGE de Hevesy (1885–1966) was one of the great pioneers of radiochemistry, perhaps the greatest. He invented many of the principal radiotracer techniques, for which he was awarded the 1943 Nobel Prize for chemistry, and the radiochemical textbook he wrote jointly with Fritz Paneth was for years the standard work on the subject. Though a chemist, he has been called “the father of nuclear medicine”, since it was to a large extent through his efforts that this field was opened up.

There is, however, surprisingly little in print about the man himself. There are three obituaries, one based almost entirely on his autobiographical notes, but not much else. It is fortunate that his long-serving assistant has given us this book.

Hevesy emerges as an enigmatic figure. He was aristocratic, courteous, reserved; one seldom talked to him about anything but science and its affairs. He took immense pleasure in his work, which is on record for all to see, but revealed little of his inner self, even in his letters to his closest friends. Life is more than science, yet Levi has been able to record no word of Hevesy's on religion or art, hardly anything on his family, and surprisingly little on political affairs considering that Nazism forced him to move from Germany to Denmark in 1934, and then to Sweden in 1943. Having a Hungarian passport, he could make these journeys freely despite being Jewish.

The book provides some illuminating sidelights on Niels Bohr, in whose Institute Hevesy spent two long and fruitful periods (1920–1926 and 1934–1943), at Bohr's personal invitation. Bohr admired Hevesy's abilities, which were very different from his own, and eagerly encouraged him to launch into the almost uncharted area of radiobiology in the second of these periods, arranging for Levi to become his assistant. She naturally describes the resultant scientific achievements in some detail, from first-hand knowledge.

Hevesy's earlier applications of radio-tracers to physicochemical problems, starting from work under Rutherford in 1911 and 1912 in Manchester, receive less attention. So long as the only tracers available were the natural radioelements, the only quantities he could measure, such as the solubility of lead chromate, were of limited interest in themselves. There was, however, great originality in his physicochemical ideas, which laid the foundations of the tremendous later developments in the use of radiotracers.

The author's command of English is generally excellent, but in places the text would have benefited from the attentions of a sub-editor. A particular point which may mislead is the use of the phrase “first of all”, not in a temporal sense, but to mean “above all” or “primarily”. Such small blemishes apart, the book tells its story well. Scientific matters are lucidly described (though the layman may find certain passages difficult even with the aid of the glossary provided) while the biographical material will be of interest to anyone concerned with the history of physics and chemistry in the twentieth century. □

Alwyn M'Kay, *Thurso, 40 Lockstile Way, Goring, Reading RG8 0AL, UK*, was formerly a Group Leader at the Atomic Energy Research Establishment, Harwell. He worked with Hevesy in Copenhagen between 1935 and 1937.



Meeting of minds — Haber's visit to Copenhagen in 1921. Standing: Neils Bohr, E. Güntelberg and I. Brønsted. Seated, G. Hevesy, Mrs A. Delbanco and Fritz Haber

Getting to grips with chaos

Michael Berry

Deterministic Chaos: An Introduction. By Heinz Georg Schuster. *Physik-Verlag*: 1985. Pp.220. DM98, \$43.75.

IN THE more fully developed sciences, natural laws are expressed as mathematical equations describing how the development of a system is uniquely related to ("determined by") its state at some other, usually earlier, time. (Quantum mechanics is no exception: its celebrated indeterminacy restricts what may be measured in any given state, not the way in which the state evolves.) Determinism does not imply predictability, however, and indeed a wide variety of systems that evolve causally, with no source of external noise, exhibit irregular and even random behaviour. The common property of deterministic chaotic systems is sensitive dependence on initial conditions: as a result of instability in the dynamical equations, two systems initially in very similar states will soon evolve to widely separated ones.

This is not a new idea. Maxwell understood it more than a century ago, and, shortly afterwards, Poincaré began to develop much of the appropriate mathematics. What is new is the emergence of "chaology" as the subject of intensive study in a variety of areas using a variety of techniques. The areas of application range from celestial mechanics (gaps in the asteroid belt) and fluid turbulence to physiological arrhythmia (heart attacks and cot deaths) and the populations in successive generations of an ecosystem. The techniques range from physical experimentation through computer simulation to the purest mathematics.

In his book, Schuster gives a very useful summary of the main ideas of the subject as it now stands. Although a physicist by training and style, he organizes his treatment by the logic of the mathematics, which is based on the concept of a dynamical system. This is any set of equations, describing how a set of variables changes with time, for which a uniqueness theorem guarantees that for each specification of the variables at one time (initial state) there is just one set of values at a later time (final state). This is a very broad framework: "time" may be continuous (as in mechanics, classical or quantum) or

discrete (as in the successive generations of an ecosystem); the set of variables may be finite (like the coordinates and velocities of a finite collection of particles) or infinite (like the triplet of velocity components at each position in a fluid).

Most of the book is devoted to simple mappings, where dynamics is reduced to a rule describing how a point in a low-dimensional space (a plane or even a line segment) jumps from "time" n to "time" $n+1$. This is a sensible strategy because much more complicated systems (such as turbulent fluids) appear to have, embedded in them, mappings which organize much of their behaviour. And astonishing complexity can indeed arise from multiple repetition of a simple map. Unraveling this has led to new concepts of wide applicability — strange attractors, Feigenbaum sequences, Lyapunov exponents, Kolmogorov entropy, for example. These and more are explained with maximum

clarity and the minimum (but subtle) mathematics; abundant illustrations, including beautiful colour plates, also help the reader along.

When there is no friction (a useful idealization in celestial mechanics and particle accelerators) different concepts apply. These are well summarized but, as the author admits, his treatment here is less original because many other accounts of this topic are available. Finally, the question of chaotic behaviour in quantum systems is discussed, with the caution appropriate to the unsettled state of the subject.

Students about to begin research into chaos, and practising scientists new to the subject, will find this a book well worth reading. □

Michael Berry is Professor of Theoretical Physics in the H. H. Wills Physics Laboratory, University of Bristol, Tyndall Avenue, Bristol BS8 1TZ, UK.

Olfactory work

D. Michael Stoddart

Social Odours in Mammals, Vols 1 and 2. Edited by Richard E. Brown and David W. Macdonald. *Clarendon*: 1985. Pp.882. Vol. 1 £45, \$60; Vol. 2 £32, \$45.

It is difficult to know whether this work is a review or a textbook — it has some of the characteristics of both. The editors say they were prompted to gather together contributions from their band of 13 authors because

the growth of theoretically exciting ideas concerning social odours, the scattered publications and expertise [gave us] the feeling that a systematic review of the whole class could provide a realistic perspective and a useful reference work.

Perhaps if they had clarified their objectives rather more they would have decided on what exactly they wanted. In my opinion *Social Odours in Mammals* is, if anything, a textbook cum reference work, although very different styles are adopted by each author. Its lack of success as a review rests largely on the poor coverage of the literature published during the current decade. Scanning the 20 bibliographies to be found at the end of each chapter one sees very few references post-1981, and most of the 1983 and 1984 references (I found only one of the latter) are to papers written by the chapter authors themselves. Book production is a notoriously slow business, but the price for delay has here been high. Olfactory biology is a rapidly developing field in which an effective review must be up to date.

The textbook quality stems from the books' rigorous taxonomic approach to the essentially functional set of phenomena referred to in the title. It is a great pity

there is no subject index (the four indexes included — author, common names, Linnean names and odour sources — occupy almost 50 pages), for there is no way, other than by reading the whole work, to find the latest ideas on, say, scent marking, or species or individual recognition. And no quick way of discovering if advances in specific areas of rodent olfactory biology, to which much space is devoted, are paralleled in other groups. This is an omission which substantially mars the books' usefulness.

What these two volumes do provide is a series of articles examining all that is known about the role of odours in mammals. The rodents are dealt with in four separate chapters — one on the effects of odour on reproductive physiology, one on the Myomorpha, one of the Sciuromorpha and one on the Hystrichomorpha. The primitive eutherians and the primates are given a different treatment; a whole chapter is devoted to a case study of the tree shrew, for example, and a chapter each to the saddle-back tamarin and to human beings. Special treatment for these and not for other well-worked species, such as the golden hamster and the Norway rat, seems inconsistent. Nonetheless, the case-histories provide excellent overviews of the work of two or three leading laboratories in the field. I also particularly enjoyed the chapters on marine mammals and the edentates and pholidotes, as these groups are seldom covered. In all chapters the treatment is thorough, and many tables enhance the presentation.

As an initiate in the field of olfactory biology I find this a useful, if somewhat eclectic, reference work. But I will direct my students to other works to gain a more rounded and up to date impression of the subject. □

D. Michael Stoddart is Professor in the Department of Zoology, University of Tasmania, Box 252C, GPO Hobart, Tasmania, Australia 7001.

The classic anatomist's textbook *The Development of the Vertebrate Skull* by Gavin R. deBeer — first published in 1937 — has just been reprinted with a new Foreword written by Brian K. Hall and James Hanken. The book is published by the University of Chicago Press. £18.95, \$22.

Build-up around the planet

John Noble Wilford

The Space Station: An Idea Whose Time Has Come. Edited by Theodore R. Simpson. *Institute of Electrical and Electronics Engineers, New York/STM Distribution, Enterprise House, Ashford Road, Ashford, Middlesex TW15 1XB, UK:1985. Pp.295. \$19.95, £18.*

COMING to terms with the proposed American space station in any rational way is little short of impossible, if only because of what it is not. Unlike the Apollo Project, it is not a programme with a specific destination (the Moon) or a clear political objective (a bold challenge to Soviet leadership in space). Unlike the Space shuttle, it lacks the coherence of a single piece of hardware already agreed on and with a single function, which was to make travel to space more routine and perhaps less expensive. (No matter that this latter objective has yet to be realized; it *was* the project's decisive selling point.) What, then, can be said of or for the space station?

In directing the National Aeronautics and Space Administration (NASA) "to develop a permanently manned space station, and do it within a decade", President Reagan in 1984 offered few details of the station's architecture, capability or purpose. He spoke only of following "our dreams to distant stars, living and working in space for peaceful, economic and scientific gain". And even though NASA had lobbied fervently for the project, its officials had no specific engineering plans ready for carrying out the Presidential order. One of their first acts was to issue contracts to industry to develop space station designs and to identify who might need or want to use such a facility. Nearly two years after President Reagan's speech, the idea has yet to be translated into firm plans.

This much can be said: the space station is supposed to be a large facility in a near-equatorial Earth orbit, about 300 km above the surface. It would be composed of several units, including a control centre and a module for housing crews or as many as ten people. These components would be launched separately from the Space Shuttle. The facility would grow module by module to meet demand for laboratories for microgravity research, materials-processing "factories", remote-sensing platforms and staging bases for missions to more distant orbits and the planets.

Some of the visions and engineering particulars for such a facility are described in *The Space Station*, which is a collection of articles by NASA officials, politicians,

science policy makers and engineers. Although the prevailing view is favourable to the principle of the space-station, the book cannot be dismissed as a propaganda tract. The editor, Theodore R. Simpson, an experienced aerospace engineer, has included several valuable discussions about the history and process of American decision-making in space matters. Several of the contributors are sceptical of the idea.

For those who want a preview of what the orbiting facility might be, two NASA engineers offer a comprehensive discussion of various designs that have been considered. In the book's illustrations, the station is usually characterized as a cluster of cylindrical modules, docking ports for shuttles and the long arms of solar-power panels. Romantics will be disappointed, for nothing resembles the toroidal station in the movie *2001: A Space Odyssey*. But as other contributors, from the Congressional Office of Technology Assessment, point out, "We would be surprised if NASA's present space station plans were carried through to completion without major change". They suggest that, in the absence of clear-cut goals, "it is simply not yet possible to judge, objectively, whether or not NASA's presently suggested infrastructure elements are truly appropriate and worth their substantial cost".

Similar reservations are expressed throughout the book. The Pentagon's lack of enthusiasm, reiterated in one chapter, must be of particular concern to space station advocates; Pentagon backing is usually essential to big-money space efforts.

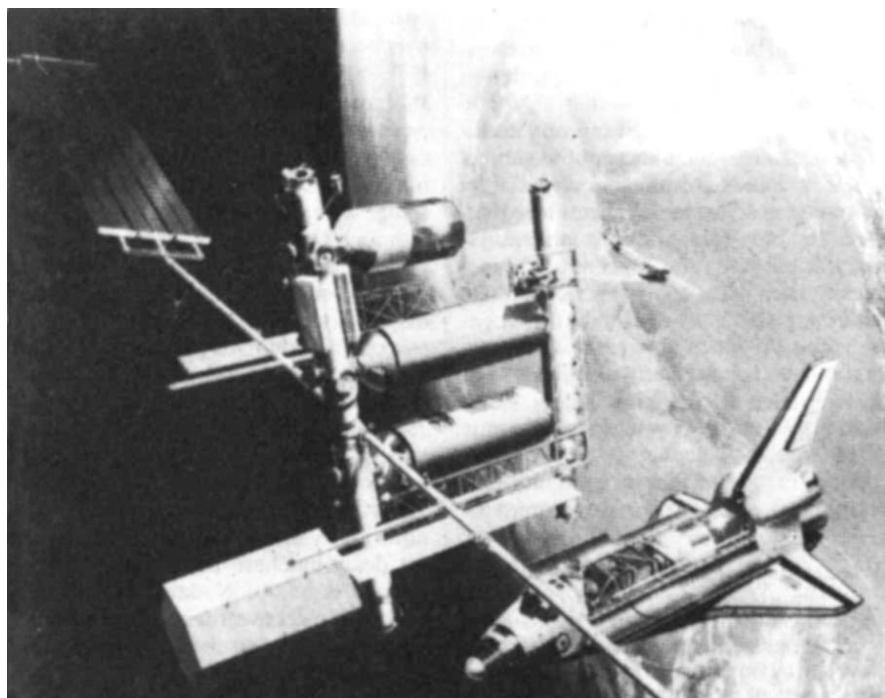
Thomas M. Donahue, a physicist at the University of Michigan, who heads the Space Science Board of the National Academy of Sciences, states the opposi-

tion of many scientists to the idea. They doubt that a space station would add significantly to what they can already accomplish with the shuttle and automated satellites. Moreover, they fear that the station's costs will drain money from their own cherished projects. But Dr Donahue seems resigned to the station's inevitability: "We hold that the decision to build a space station will be made for reasons other than its usefulness to space science and applications".

Victor M. Reis, a former science policy adviser in the Reagan Administration, takes this point even further. He acknowledges that each and every one of the projected space station missions could be done well by a judicious combination of shuttles, Spacelabs, automated platforms and satellites. Nonetheless, he argues that there is one "powerful argument" in support of the project, which is, in effect, to assure NASA of a robust future. Mr Reis is probably correct in calling this "the fundamental issue" of the space station decision. But he cautions that a decision to go forward with the project would be justified only if "it can be done in harmony with NASA's other missions and responsibilities". This will not be easy, he notes, because NASA's big manned programmes have a way of consuming the lion's share of NASA's budgets.

The Space Station will not be satisfying to those who seek to win converts to the space-station cause or to those who are its outright opponents. More than most of the authors may have intended, the book reveals why the project has so far had such a cool to mixed reception. □

John Noble Wilford is a reporter on science and space for the New York Times.



Not yet a reality — the concept of a Space Operations Center developed by NASA in 1979.

Sociological problems of high-energy physics

from Andrew R. Pickering and W. Peter Trower

Large-scale collaborative projects between experimental high-energy physicists have produced sociological problems that will need thought to resolve.

TEAM research entered the study of elementary particles in the immediate aftermath of the Second World War. The collaborative methods which had borne fruit in the Manhattan Project were carried over into the new speciality of high-energy physics. The most visible early manifestation of this organizational transfer was at the Radiation Laboratory of the University of California, Berkeley, where E.O. Lawrence's pre-war development of the cyclotron had led to a central role in atomic weapons research¹. As R.R. Wilson, the first director of the Fermi National Accelerator Laboratory, recalled in 1970, "A word was even coined — Berkeleitis — to describe the syndrome that existed there"².

Despite the misgivings of physicists who harked back to the pre-war days of individualistic research, Berkeleitis soon became endemic to high-energy physics, growing more rampant with the passing years³. Until the mid-1960s it was still possible for a small group of, say, less than ten physicists to make a significant contribution to high-energy physics; by the late 1970s the complement of a typical collaboration had grown to around 50. Today, groups of around 200 physicists are assembling in preparation for experiments at Europe's next new big machine — the Large Electron-Positron Collider (LEP), due to come into operation at CERN, Geneva, in 1987.

As collaboration size has grown, so too has the duration of typical experiments. In the 1960s an experiment could be mounted and data analysed within a period of months, while current experiments can span more than a decade from first conception to final publication of results. Thus, in terms of both personnel and duration, high-energy physics experiments have moved ever further from the pre-war stereotype of the lone researcher at the laboratory bench, and have come more to resemble large-scale engineering projects.

Trends

The underlying trend towards larger collaborations and extended timescales in experimental high-energy physics is not hard to understand. The relatively generous funding which high-energy physics has enjoyed throughout its history has made possible the construction of a succession of particle accelerators of ever-increasing size, and sophisticated experimental techniques have been developed to exploit the

high-energy beams thus made available. Instead of a single detector, a modern experiment deploys a complex multi-element array, costing millions of dollars and requiring an army of specialists to design, build and run. Considerable resources are also required for subsequent data analysis.

Neither is it hard to appreciate the benefits of this form of research. The discoveries at CERN of the electroweak intermediate vector bosons, W^+ and Z^0 , made by two groups totalling nearly 200 physicists, were the culmination of a decade of rapid progress in understanding elementary-particle interactions. But such benefits have been bought at a price. Our aim here is to outline the sociological problems which are the symptoms of Berkeleitis. Our discussion is organized around twin themes: the frustration of individual initiative and creativity within large collaborations, and the consequent tendency to conservatism and orthodoxy in communal practice. (The frustrations inherent in team research have been impressed upon us in informal communication with many experimenters. It seems clear that the present trend towards non-accelerator experiments (for example, searches for free quarks, magnetic monopoles, proton-decay) is, in part, a manifestation of these frustrations.) We will show that the problems arising within individual experiments spill over into programmes of experiment. They are reinforced by the institutional structure of experimental high-energy physics, and have been further exacerbated by conceptual developments in the field over the past decade. Finally, we ask whether anything can be done to check current trends to gigantism and orthodoxy in high-energy physics research.

The rationale for team research is the division of labour. A large collaboration comprises several groups of physicists, each drawn from a single university or laboratory. In setting up and running an experiment, each group takes on responsibility for particular elements of the overall apparatus. Data analysis is likewise broken down into component tasks allotted to different groups. Thus, along with the division of labour goes a degree of specialization beyond that normally associated with scientific research.

This is where sociological problems begin to arise, particularly for junior physicists — postgraduate students and young postdoctoral researchers. Even within a

single group of experimenters, research tasks are subdivided. And junior physicists are usually those responsible for the basic items of hardware and software. They thus acquire detailed knowledge of only one small part of an enormous and diversified project, and their chances of making a significant contribution to the overall course of the experiment outside their area of expertise are slight.

Managers

Senior physicists encounter a similar problem, but for different reasons. They oversee and coordinate the work of a group or groups, and are thus obliged to take on administrative responsibilities more familiar to managers of large technological enterprises. Another function of senior physicists is to secure the material resources necessary for the success of the experiment, and to negotiate with laboratory managements for beam-time, workspace and so on. Here they function as entrepreneurs. Again, even at the most senior level, the role of creative scientist can be submerged for long periods of time (if not forever) by the multifaceted organizational demands of a large collaboration.

In a variety of ways, then, many physicists find their creative ambitions and aspirations frustrated in the course of high-energy physics experiments. And, all things being equal, opportunities for innovation decrease in proportion to the degree of specialization and the associated administrative load — that is, in proportion to collaboration size and the duration of the experiment.

Only in a field where long experiments were commonplace, though, would one even look for creative opportunities within the development of a single experiment. The classic locus for creativity in experimental science lies in the gaps between experiments: in the conception of new techniques or new problems to tackle. But here too Berkeleitis has a deadening effect. One can discern at least two sources of conservatism in experimental design in high-energy physics. The first is that specialization is self-reproducing. As discussed above, in his research training, the young physicist acquires a narrow, specialized competence in some limited aspect of experimental high-energy physics. This competence then becomes his most precious asset in the pursuit of a research career, so valuable (to himself and to others) that opportunities for future variety in research practice are highly

circumscribed. And the upshot of this is an inhibition of technical innovation within high-energy physics experiment as a whole.

Since techniques are usually appropriate to the exploration of a limited class of phenomena, technological conservatism itself implies conservatism in the choice of research problems, and both forms of conservatism are reinforced by a second source: the fear of failure. The senior physicist, or physicists, who propose to conduct a contemporary high-energy physics experiment risk not only their personal reputations, but also the efforts and careers of the many less senior physicists who will be involved in the project. If the experiment fails — if it does not produce interesting data or, indeed, any data at all — hundreds, possibly thousands, of man-years of effort and millions of dollars will have been wasted. Not surprisingly, therefore, there is a tendency to conservatism in experimental programmes, with new experiments aiming to investigate phenomena of well-established interest using well-established techniques.

Rewards

So far we have outlined the obstacles to initiative and creativity implicit in collaborative research of long duration: the twin requirements of specialization (with its self-reproducing character) and administration, and the ever-present risk of failure. In themselves, the impact of these factors can be overestimated. Against them must be set the potential rewards for successful innovation — symbolic rewards like Nobel prizes, and material rewards such as career advancement. There are, however, institutional factors in high-energy physics which serve to structure even successful innovations, to foster a limited set of technological developments or problem choices at the expense of others. This, too, is a form of conservatism, which again acts to stifle individual initiative.

The origins of institutional conservatism in high-energy physics lie in the centralization of research resources. Despite generous funding, the expense of building and running particle accelerators has long been beyond the resources available to individual universities. Over the history of the field, the facilities for high-energy physics experiments have been gathered together into a handful of regional, national and international laboratories at which collaborations assemble to perform their experiments before departing to their home universities for data analysis.

Together with the centralization of research resources has gone a proliferation of committees. Access to experimental beams and funding for experiments are controlled by committee, likewise planning and funding for new facilities — new accelerators or new major items of experimental equipment. Thus any potential innovator is faced with an institutional

hurdle. The typical disposition of committees towards conservatism is well known, and the experimenter who does not propose to tackle an established problem with established techniques is likely to find his proposal rejected.

As Nobel Laureate Luis Alvarez put it: "Our present scheduling procedures almost guarantee that nothing unexpected can be found"⁴. As it happens, there is a twofold irony to this quotation. In his leadership of the 72-inch bubble chamber programme at Lawrence's Radiation Laboratory, Alvarez did more than any other to demonstrate the benefits of Berkeleyitis in experimental high-energy physics⁵. (For a discussion of the Alvarez group, see ref. 6.) And at the time when he wrote, 1973, high-energy physics was characterized by a pluralism of research strategies that was soon to vanish.

The 1970s saw the rapid development of the 'new physics' world-view in high-energy physics, in which the world was seen to be built from quarks and leptons interacting according to the dictates of twin gauge theories — quantum chromodynamics (for the strong interaction) and the Weinberg-Salam-Glashow unified electroweak theory. And the sociological correlate of the 'new physics' was the 'new orthodoxy' (as it was christened by high-energy physics theorist James Bjorken)⁷. Within the new orthodoxy, communal research practice in high-energy physics became almost exclusively organized around the new-physics world-view. The effect was most striking in the experimental fields, where the institutional committee structure reflected the majority view and effectively enforced the new-physics dominance of research. Since the late 1970s it has been difficult, if not impossible, to mount a high-energy physics experiment which does not promise to engage directly with the interests of gauge theorists.

One consequence, then, of the rise of the new orthodoxy has been a close circumscription of opportunities for initiative in the choice of topics for experimental investigation. Along with this restriction on problem choice have gone restrictions on acceptable forms of technical innovation. As currently elaborated, gauge theory offers an analysis of only a limited range of rare phenomena. Investigation of those phenomena (in the presence of an overwhelming background of 'uninteresting' processes) requires large, complex experiments and highly sophisticated electronics — precisely the experiments which call for large collaborations working together over a period of years. Larger and larger teams have assembled to man the ever-larger experiments expected to probe the fine details of already rare phenomena, leading to the projection of 200 member teams for LEP.

Thus, more than any other factor, it has been the new physics (via the new orthodoxy and the institutional structure of

high-energy physics) which has driven the trend to gigantism in experimental physics throughout the 1970s and into the 1980s. And, as an unintended consequence, it has multiplied all of the sociological problems discussed above. The degree of specialization in the experimental physics community, the extent of administrative responsibilities and the risks attached to failure have all increased with the coming of the new physics. Opportunities for initiative and creativity have decreased in proportion.

The new physics, then, through the grip of the new orthodoxy on experimental high-energy physics, is experienced by many physicists as making the life of the researcher more frustrating and less rewarding. What of the future? At present, the new orthodoxy, with its attendant drive to gigantism and the multiplication of sociological problems, is set to reproduce itself indefinitely. Through the institutional structure of high-energy physics, the future as well as the present of experimental research has been given over to the new physics. The next generation of big machines — at which the next generation of experimenters will learn their trade — are explicitly conceived as new-physics facilities. LEP, for example, is intended to operate as an intermediate vector boson factory (see, for example, ref. 8).

The state of high-energy physics is therefore not entirely satisfactory, despite the conceptual triumphs of the new physics. The existence of a self-perpetuating orthodoxy is anathema to many physicists, especially an orthodoxy which multiplies pre-existing sociological problems by further stifling opportunities for individual initiative. The question arises: can anything be done to ameliorate this situation? Clearly, a return to the pre-war days of the lone researcher is impossible — Berkeleyitis is intrinsic to the technical and institutional fabric of experimental high-energy physics — but can the slide into gigantism be checked (for those who wish it)?

Here there is a straightforward suggestion. The institutions of high-energy physics should be used to relax rather than to enforce the stranglehold of the new physics upon experiment. A fraction of the available resources should be set aside for those who would, for one reason or another, follow a heterodox path. In this way, the oppressive aspect of the current orthodoxy could be *de facto* eradicated. And, since it is the new-physics emphasis on rare phenomena which is driving the present increase in collaboration size and experiment duration, the route would be open for individualistically inclined researchers to move towards their chosen form of practice. Unconventional paths of inquiry could be followed, novel small-scale experimental techniques could be developed and old techniques rescued from oblivion, all with unpredictable but possibly major consequences for future

patterns of research.

It is important to stress that the argument is not that the new-physics worldview should be abandoned — its virtues are too well established for that — but simply that some opportunities should be left for research outside the orthodoxy. (It is worth recalling that it was precisely such heterodox research that led to the 1974 discovery of the J-psi particle and the establishment of charm — an episode that marked a watershed in the development of the new physics.) Of course, in comparison with a well-established orthodoxy, heterodox experimental proposals inevitably appear lightweight — perhaps frivolous or even incomprehensible — and committees find it correspondingly hard to support them. But this point has already been adequately dealt with by the mathematical physicist Freeman Dyson, who suggests that funding agencies should allot somewhere in the region of 10–25 per cent of their resources to heterodox research. (The figure of 10 per cent is also suggested by Muller. Alvarez argues that proposals in experimental high-energy

physics should be assessed solely on the basis of the experimenters' past performance, and without regard to consensually perceived theoretical significance.)

It should be noted that although this article has focused upon sociological problems arising in experimental high-energy physics, problems also exist in theory. Here again institutional structures presently act to discourage practice outside the gauge-theory orthodoxy. Dyson's article is, in fact, concerned with encouraging theoretical rather than experimental diversity. His conclusion is, however, relevant to both theoretical and experimental practice: "We should not be afraid of looking foolish or even crazy. We should not be afraid of supporting risky ventures which may fail totally . . . Organizations which only support research where there is no chance of mistakes will in the end support only mediocrity. If we proceed with good sense and courage to support unfashionable people doing things that orthodox opinion considers irrelevant or crazy, there is a good chance that we shall rescue for science . . . people

whose ideas will still be famous long after all our contemporary fashionable excitements are forgotten".

This work was supported in part by the Social Science Research Council, UK (A.R.P.), NSF-PHY-7913184 (W.P.T.) and the Jeffress Foundation (W.P.T.). □

Andrew R. Pickering is at the Department of Sociology and Program in Science, Technology and Society, University of Illinois, Urbana, IL 61801, USA, and W. Peter Trower is at the Department of Physics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA.

1. Seidel, R.W. *Historical Studies in the Physical Sciences* 13, 375 (1983).
2. Wilson, R.R. *Daedalus*, Fall, 1076 (1970).
3. Morrison, D.R.O. in *Physics from Friends: Papers Dedicated to C. Peyrou on His 60th Birthday* (eds Armenteros R., et al.) 351–365 (Multi Office, Geneva, 1978).
4. Alvarez, L.W. *Adventures in Experimental Physics* Vol 3, v–vii (1973).
5. Alvarez, L.W. in *Nobel Lectures Physics, 1963–1970*, 241–290 (Elsevier, London, 1972).
6. Swatez, G.M. *Minerva* 8, (1), 37 (1970).
7. Bjorken, J.D. in *Proceedings of Neutrino 79* (eds Haatuft, A. & Jarlskog, C.), 9–19 (Bergen, Norway, 1979).
8. Report of the LEP Study Group *Design Study of a 22 GeV to 130 GeV e^+e^- Colliding Beam Machine (LEP)* (CERN 'Pink Book' ISR-LEP/79-33).
9. Dyson, F.J. *The Mathematical Intelligencer* Vol. 5, 47 (1983). Muller, R.A. *Science* 209, 880 (1980). Alvarez, *op.cit.*, note 4.

ARTICLES

Concentration of methane in the troposphere deduced from 1951 infrared solar spectra

Curtis P. Rinsland, Joel S. Levine & Thomas Miles

Atmospheric Sciences Division, NASA Langley Research Center, Hampton, Virginia 23665, USA

The 1–2% increase in tropospheric methane determined from gas chromatographic measurements since 1977 has important implications for atmospheric photochemistry and climate. To extend the measurement baseline, we have analysed solar absorption spectra and determined mean troposphere CH_4 mixing ratios for the years 1951 and 1981, and find an average rate of CH_4 increase of $1.1 \pm 0.2\%$ per year for this period.

ALTHOUGH methane is only a trace atmospheric constituent at the part per million by volume (p.p.m.v.) level, it has a significant impact on the photochemistry of both the troposphere and stratosphere and on the Earth's climate. In the troposphere, the methane oxidation chain initiated by the reaction of CH_4 with the hydroxyl radical (OH) leads to significant photochemical production of H_2 , CO and O_3 (ref. 1). In the stratosphere, CH_4 is a source of odd hydrogen species (HO_x) which catalytically destroy O_3 (ref. 2). In addition, CH_4 reacts with atomic chlorine in the stratosphere, terminating the odd chlorine cycle, which catalytically destroys O_3 (ref. 2). Methane also contributes to the atmospheric greenhouse effect by its absorption in the 7–14- μm spectral region³.

Over the past 5 years, several studies have reported that levels of CH_4 in the troposphere may be increasing. The analysis of urban or suburban total hydrocarbon data in New Jersey over 10 years (1968–1977) led to the suggestion that CH_4 (which was not monitored explicitly, but is the major component of the measured total hydrocarbon concentration) may have increased at the rate of $\sim 0.6\%$ yr^{-1} (ref. 4). Analysis of almost 1,600 nearly continuous measurements of CH_4 obtained over a 22-month period (January 1979–October 1980) at Cape Meares, Oregon, indicated that CH_4 was increasing at a rate of $1.9 \pm 0.5\%$ yr^{-1} (ref. 5). A similar increase was found in measurements over

western Europe from 1977 to 1981 (ref. 6). Measurements of CH_4 between November 1977 and November 1980 in remote locations between 55°N and 53°S indicate a 1% yr^{-1} increase in CH_4 between January 1978 and January 1980 (ref. 7). Additional Cape Meares measurements obtained between October 1980 and December 1981, as well as 3 yr of measurements from the Arctic Circle to the South Pole, suggest a global increase of $1\text{--}1.9\%$ yr^{-1} (ref. 8). The results of >800 CH_4 measurements over southern Australia (September 1980–March 1983), from the surface to the upper troposphere, show an average rate of tropospheric CH_4 increase of 1.3% yr^{-1} at December 1981 (ref. 9). Three different sets of CH_4 measurements were examined for trends by Ehrlach *et al.*¹⁰: (1) 49 tropospheric profiles obtained from 1965 to 1967 and 1971 to 1974 by aircraft flights at the National Center for Atmospheric Research (NCAR); (2) measurements obtained from 1968 to 1979 from ships and aircraft by the Naval Research Laboratory (NRL); and (3) published measurements of the total atmospheric CH_4 column amount deduced from ground-based infrared spectra recorded between 1948 and 1975. Ehrlach *et al.*¹⁰ summarized their conclusions from these three data sets, as well as the other measurements of CH_4 , in the following way: (1) There was little or no increase in CH_4 between 1948 and 1965, shown by published analyses of the ground-based infrared spectra. (2) NCAR

and NRL measurements and the data of Graedel and McRae⁴ indicate a 0.5% yr⁻¹ increase in CH₄ between 1965 and 1975. (3) There is good evidence for a global CH₄ increase of ~1–2% yr⁻¹ between 1978 and 1980 based on several sets of measurements^{5–8}.

Ehhalt *et al.*¹⁰ also discuss the analyses of air trapped in Greenland and Antarctic ice from 100 to 2,500 yr ago, which yield significantly lower levels of CH₄ (refs 11–13). Ehhalt *et al.*¹⁰ suggest that these low CH₄ levels are caused either by lower levels of atmospheric CH₄ at the time the air was trapped in the ice or by trapped CH₄ that may have been lost by diffusion through the ice. They conclude that the relation of CH₄ levels in ice to the atmospheric CH₄ levels of the past remains unclear.

Although the published ground-based infrared spectral measurements indicate little or no increase in CH₄ in the troposphere between 1948 and 1965 (ref. 10), these early studies were limited in their accuracies by uncertainties in the assumed values for the spectroscopic line parameters, the vertical CH₄ profile shape, the vertical pressure–temperature profile and the relatively simple methods of analysis available at that time. To obtain a more accurate determination of the early CH₄ tropospheric concentration, we initiated a study to deduce the levels of tropospheric CH₄ and other gases from such historical spectra.

Here we report an analysis of infrared solar absorption spectra recorded in April 1951 at the Sphinx observatory of the Jungfraujoch International Scientific Station in the Swiss Alps (latitude 46.5° N, longitude 8.0° E; elevation 3,578 m)¹⁴. We have used the best available line parameters for CH₄ and interfering lines of H₂O in the analysis, and determined the average tropospheric mixing ratio of CH₄ from a line-by-line, multi-layered atmospheric, nonlinear least-squares spectral fitting procedure. Correlative radiosonde and surface pressure–temperature measurements were used to determine the vertical pressure–temperature profile for the analysis. A mean tropospheric mixing ratio of 1.14 ± 0.08 p.p.m.v. has been deduced for CH₄ from the analysis. As uncertainties in line parameters remain a problem in interpreting spectroscopic data, we also derived the mean concentration of CH₄ in the troposphere from analysis of the same region in Kitt Peak solar spectra taken in February 1981. From the derived value of 1.58 ± 0.09 p.p.m.v., which is close to other modern determinations, and our value for April 1951, we determine an average rate of increase of 1.1 ± 0.2% yr⁻¹ for 1951–81.

Detailed spectral analysis is the key to deducing the concentrations of trace species in the troposphere from historic solar spectra. Hence, most of this paper deals with the spectral analysis that led to the determination of a significantly lower tropospheric concentration of CH₄ in 1951, and with the reconciliation of this new, lower value with the older, higher spectral-deduced values.

Observations and spectral analysis

The Jungfraujoch measurements selected for analysis were made with a coelstat and Pfund-type prism-grating spectrograph on the morning of 15 April 1951, between 09 h 59 min and 10 h 17 min UT. These data cover the 2,901–2,926-cm⁻¹ spectral interval and have been published as part of an infrared photometric atlas¹⁴. According to Table 2 of the atlas, the zenith angle during the measurements decreased from 41.5 to 39.8°. The infrared signal was detected by a Perkin-Elmer thermocouple together with a 13 c.p.s. chopping system and amplifier manufactured by the same firm and connected to a Leeds and Northrup type-G Speedomax recorder. A grating with a 16 × 12 cm ruled surface and an effective slit width of 0.24 cm⁻¹ was used in the region considered here. Additional details of the instrumentation are given in the atlas¹⁴ and elsewhere^{15,16}.

Absorption in the analysis interval is dominated by lines of CH₄ with minor contributions from water vapour and solar lines. The spectral data were digitized from the atlas using a Houston Instruments Hipad digitizer attached to a model PGM-2 computer (Superset). The zero-signal level indicated in the atlas has been assumed. The wavenumber scale of the digitized data has been calibrated based on measurements of the line centre posi-

Table 1 Some atmospheric parameters assumed in the analysis of Jungfraujoch spectra

Layer boundaries (km)	Temperature (K)	Pressure (mbar)	CH ₄ mixing ratio (p.p.m.v.)	
			Case A	Case B
3.578–5.0	255.9	594.0	1.60	1.60
5.0–7.5	243.2	456.2	1.60	1.60
7.5–9.0	228.1	339.0	1.60	1.60
9.0–12.0	216.7	244.8	1.60	1.60
12.0–15.0	215.6	152.4	1.55	1.55
15.0–18.0	216.2	95.1	1.43	1.43
18.0–20.0	216.2	63.7	1.25	1.32
20.0–22.0	216.3	46.7	1.10	1.26
22.0–24.0	216.7	34.2	1.00	1.20
24.0–26.0	219.0	25.0	0.90	1.10
26.0–28.0	222.8	18.4	0.83	1.00
28.0–30.0	226.7	13.6	0.75	0.90
30.0–32.0	231.9	10.1	0.67	0.80
32.0–35.0	240.1	7.13	0.58	0.70
35.0–40.0	252.4	4.25	0.45	0.60
40.0–60.0	265.2	1.54	0.25	0.38
60.0–100.0	233.6	0.10	0.05	0.05

Total vertical column amount of air molecules 1.380×10^{25} cm⁻². Altitude of the tropopause ≈ 10.2 km.

tions of 13 absorption features distributed nearly evenly in wavenumber throughout the region. The centre positions were obtained with a line-finding algorithm similar to that used in a recent analysis of CO₂ laboratory spectra¹⁷. For unblended lines, the wavenumbers from the 1982 Air Force Geophysics Laboratory (AFGL) major gas compilations¹⁸ were adopted. For blended features, a strength-weighted mean position from the 1982 AFGL major gas compilation¹⁸ was adopted initially; this value was refined by measuring the line centre position of the feature in a preliminary least-squares best-fit calculated spectrum. Linear interpolation was used to determine the wavenumbers of data between the calibration points.

Three sources of meteorological information were used in the determination of the profile of pressure and temperature as a function of altitude above the Jungfraujoch station on 15 April 1951: (1) surface measurements of pressure (*P*) and temperature (*T*) at the Jungfraujoch station on 15 April, interpolated for the time the infrared spectrum was recorded (P. Hachler, personal communication); (2) tropospheric radiosonde *P*–*T* soundings from Payerne (46.5° N, 6.6° E) for 05 h UT on 14 April and 16 April (P. Hachler, personal communication); and (3) stratospheric–mesospheric climatological *P*–*T* profiles for April derived from a composite of 5–20 yr of daily radiosonde and satellite measurements for the mid-latitude Northern Hemisphere^{19,20}. The above information was used to define the *P*–*T* profile up to the upper mesosphere; above this altitude, mid-latitude standard atmosphere values for spring were assumed²¹.

The tropospheric portion of the *P*–*T* profile was derived by first interpolating the two Payerne radiosonde profiles to the time and date of the spectroscopic measurements. Not surprisingly, the interpolated values corresponding to the altitude of the Jungfraujoch station (650.5 mbar and –12 °C) differed slightly from the surface measurements (655 mbar and –14 °C). To improve the compatibility of these two sources of meteorological information, we adopted the measured surface values and adjusted slightly the altitudes corresponding to the interpolated upper air *P*–*T* measurements, using the hydrostatic equation. A standard meteorological thermodynamic diagram was used for this purpose.

The 17-layer model adopted in the analysis of the Jungfraujoch spectrum is presented in Table 1. The airmass per layer was calculated with the FSCATM ray-tracing program from FASCOD1C²², assuming the *P*–*T* profile discussed above and the mean solar zenith angle given for the observations¹⁴. The pressures and temperatures listed for each layer are effective values weighted by the density of air along the refracted atmospheric path.

Ground-based solar absorption spectra contain only very limited information about the altitude distribution of atmospheric gases. Therefore, we have assumed a relative mixing-ratio profile for both CH_4 and H_2O based on reported measurements. The absolute values are derived by scaling the relative mixing ratio in each layer by a single multiplicative factor determined for each gas from the spectroscopic analysis.

As no measurements of the vertical distribution of CH_4 were made in 1951, calculations have been performed assuming two profile shapes based on recent data (Table 1). For both cases, we have adopted a constant mixing ratio of 1.60 p.p.m.v. below 12 km. The stratospheric portion of the case A profile is representative of CH_4 measurements made between 40°N and 60°N using *in situ* and remote techniques, including the results of the NIMBUS 7 Stratospheric and Mesospheric Sounder (SAMS) instrument. It has been obtained by drawing a smooth curve through the values presented in Fig. 7 of ref. 23. The case B profile assumes higher mixing ratios in the upper stratosphere than were usually observed by NIMBUS 7 SAMS at 40°N – 60°N . It is representative of results from the same data sources for the 20°N – 40°N latitude range and has been derived in the same way from Fig. 6 of ref. 23. The case B values have also been used in the analysis of the 1981 solar spectra from Kitt Peak (32°N). The 1962 US Standard Atmosphere water vapour distribution²⁴ has been adopted for the H_2O profile shape. High-resolution ground-based spectra show lines of NO_2 in this spectral region^{25,26}. Although these lines are too weak to be identified in the Jungfraujoch spectra, we have included them in the radiative calculations assuming a published NO_2 reference profile²⁷.

Line parameters for CH_4 , H_2O and NO_2 were taken from the 1982 AFGL major and trace gas compilations^{18,28}. A T^{-n} temperature dependence was assumed for the air-broadened half-widths with values of $n=0.9$ for CH_4 , $n=0.5$ for H_2O and $n=0.92$ for NO_2 . The value for CH_4 is based on several sets of laboratory measurements^{29–31} that have found values of n near unity for self-, N_2 - and air-broadening. The value for NO_2 is taken from a recent study on the N_2 broadening of lines in the ν_3 and $\nu_2 + \nu_3 - \nu_2$ bands³². The value of $n=0.5$ for H_2O corresponds to the assumption of a temperature-independent collision diameter.

The analysis using the technique of nonlinear least-squares spectral curve fitting follows the procedures described recently for the interpretation of high-resolution ground-based spectra^{33,34}. This method has proven particularly suitable for the present study because of its accuracy in establishing the background level and its usefulness in regions of overlapping absorption lines, as observed in the Jungfraujoch data. In this study, a total of four parameters were adjusted simultaneously in the least-squares fittings to the digitized spectrum (point spacing = 0.02 cm^{-1}). Two of the parameters model the instrument response: one is for the background level of the spectrum and the other is used to fit the instrument line-shape function. Gaussian slit functions have been found for two representative grating spectrometers³⁵. We assumed this line shape in our analysis with the width of the gaussian function as an adjustable parameter in the least-squares fits. The other two parameters are the multiplicative scaling factors that convert the relative profiles of CH_4 and H_2O to absolute values.

Two sections of the digitized Jungfraujoch data were analysed assuming the case A profile shape for CH_4 . From the $2,903.0$ – $2,912.2\text{ cm}^{-1}$ region, a total vertical column amount of 1.510×10^{19} molecules cm^{-2} is deduced for CH_4 , corresponding to a tropospheric mixing ratio of 1.16 p.p.m.v. From the $2,915.2$ – $2,922.0\text{ cm}^{-1}$ region the corresponding values are 1.471×10^{19} molecules cm^{-2} and 1.13 p.p.m.v. The average of these two determinations of the CH_4 tropospheric mixing ratio (1.14 p.p.m.v.) is considerably lower than modern values^{5–9}. Fits with the case B CH_4 profile shape produced tropospheric CH_4 mixing ratios only 0.3% smaller than the case A values. The small magnitude of this difference results from the fact that only a small percentage of the total number of CH_4 molecules along

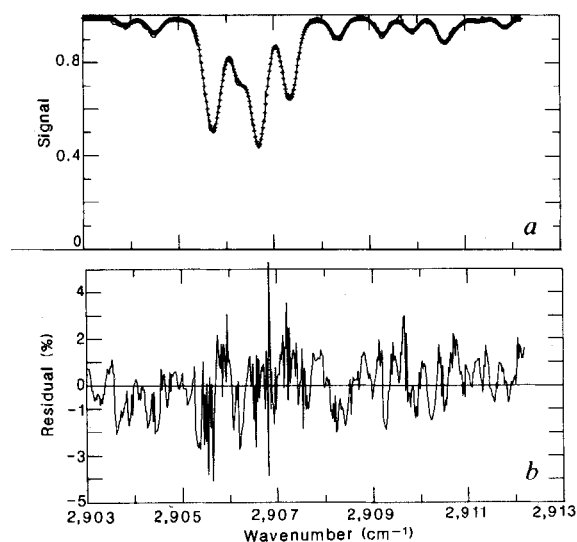


Fig. 1 Comparison (a) between the Jungfraujoch digitized spectrum (solid line) and a least-square best-fit to the data (plus symbols). Residuals (b) are observed – calculated values.

the atmospheric path are located in the middle and upper stratosphere where the two assumed profile shapes are different. Slightly larger changes in the tropospheric CH_4 mixing ratio were obtained by modifying the assumed profile shape in the lower stratosphere. For example, if the case A profile shape is assumed to be above 18 km and 1.60 p.p.m.v. is assumed for all altitudes below 18 km, tropospheric CH_4 mixing ratios 0.7% lower than the case A values are calculated.

As noted above, uncertainties in the assumed line parameters remain a major potential source of error in spectroscopic retrievals. Therefore, as a check on both our analysis procedure and the spectroscopic parameters that we have adopted, the CH_4 tropospheric mixing ratio has also been determined from modern data covering the same spectral region. For this purpose, we have selected a particularly dry spectrum recorded at an average solar zenith angle of 70.65° on 23 February 1981 from Kitt Peak (latitude 31.9°N , longitude 111.6°W ; elevation 2,095 m). The observations were made at 0.01 cm^{-1} resolution with the Fourier transform interferometer in the McMath solar telescope complex which is operated by the National Solar Observatory (see ref. 34 for details). To increase the validity of the comparison between the old and new data, the resolution of the Kitt Peak spectrum has been degraded by convolving the measured data with a normalized gaussian function having a width (cm^{-1}) equal to that retrieved from the Jungfraujoch spectral analysis. A correlative P – T profile based on radiosonde and global satellite measurements³⁶ has been assumed in the ray-tracing calculations. A tropopause height of 10.4 km is indicated from these results.

Assuming the case B relative mixing-ratio profile for CH_4 in the stratosphere (which is representative of measurements for the latitude of Kitt Peak), the total vertical column amount and concentration of CH_4 in the troposphere determined from the Kitt Peak spectra are 2.533×10^{19} molecules cm^{-2} and 1.56 p.p.m.v. from the $2,903.0$ – $2,912.2\text{ cm}^{-1}$ region and 2.608×10^{19} molecules cm^{-2} and 1.60 p.p.m.v. from the $2,915.2$ – $2,922.0\text{ cm}^{-1}$ region. The average of these two determinations of the CH_4 tropospheric concentration in February 1981 is 1.58 p.p.m.v., slightly lower than published *in situ* measurements for the same time and at nearly the same latitude^{6–8}. However, within our estimated experimental uncertainty of 6% (discussed below), there is agreement in the results.

The results of the fits obtained for the Jungfraujoch and Kitt Peak spectra in the $2,903.0$ – $2,912.2\text{ cm}^{-1}$ region are shown in Figs 1 and 2. The measured intensities have been normalized to the highest value in the fitted region, and the residuals

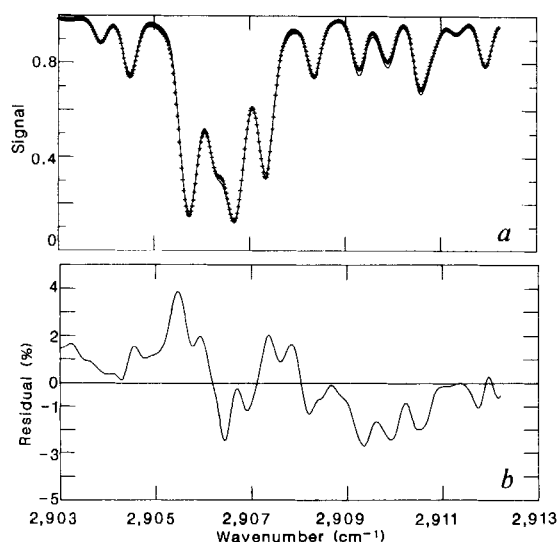


Fig. 2 Comparison (a) between the degraded Kitt Peak spectrum (solid line) and a least-squares best-fit to the data (plus symbols). Residuals (b) are observed-calculated values.

(observed minus calculated) are expressed as a percentage of the peak measured intensity. The standard deviations for the Jungfraujoch and Kitt Peak spectral fits are 1.19 and 1.44%, respectively. Although the agreement between the observed and best-fit calculated spectra is good for both data sets, some minor systematic discrepancies can be noted, especially for the higher air mass and higher signal-to-noise Kitt Peak data. The quality of the fitting results is about the same for the 2,915.2–2,922.0 cm^{-1} region.

Error analysis

A plot on an expanded scale of the observed and least-squares best-fit calculated spectra shows that a very good fit to the Jungfraujoch instrument function has been obtained with the gaussian line-shape model adopted in the analysis. However, it is important to test the sensitivity of the results to possible deviations from this model. Therefore, we repeated the Jungfraujoch retrieval for the lower wavenumber region assuming triangular and lorentzian line-shape functions. Again, a single adjustable width parameter was included in the analysis. Values for the CH_4 tropospheric mixing ratio and the standard deviation of the fit were, respectively, 1.15 p.p.m.v. and 1.26% for the triangular function and 1.24 p.p.m.v. and 1.70% for the Lorentz function, compared with 1.16 p.p.m.v. and 1.19% for the gaussian function. Plots of the results showed that the quality of the fits were nearly the same for the gaussian and triangular response functions, but that the strong wings modelled by the Lorentz function clearly did not represent satisfactorily the observational data. From these tests, we estimate that at most a 3% error in the retrieved mixing ratios can be caused by uncertainties in the line-shape function.

Scattered light and any nonlinearities in the detector-amplifier system are important sources of potential error. Both these effects are discussed in the solar atlas¹⁴, and produce negligible errors in the recording of the spectral data. The zero level was recorded at the beginning of the short section of spectrum analysed in this study. Therefore, the error in the zero level is also believed to be negligible. Although a number of solar lines can be identified in the 0.01- cm^{-1} resolution Kitt Peak spectra, their contribution to the total absorption in the analysed region is small and negligible error results from excluding them from our spectral simulation/retrieval algorithm.

In addition to the sensitivity studies already discussed, calculations similar to those presented in two previous studies^{34,36} were made to estimate the uncertainty in the CH_4 tropospheric mixing ratio caused by uncertainties in key parameters and by instrument noise. The parameters considered are the CH_4 line

Table 2 Systematic error sensitivity study

Error parameter	CH_4 tropospheric mixing-ratio error (%)	
	JF	KP
a, $\Delta S/S = +0.05$ for all CH_4 lines	-5	-5
b, $\Delta b_c/b_c = +0.05$ for all CH_4 lines	-2	-3
c, $\Delta n/n = +0.20$ for all CH_4 lines	-2	-2
d, $\Delta T = +2$ K for all layers	-1	-1
e, $\Delta P/P = +0.02$ (JF), $+0.01$ (KP) for all layers	-2	-1
f, 1.1% (1σ) gaussian instrument noise (JF only)	1	<1
g, Instrument line shape	3	<1
h, Vertical profile shape	<1	<1
i, r.s.s. of a–h	7	6

JF, Jungfraujoch; KP, Kitt Peak.

intensities S , the CH_4 air-broadened halfwidths at 296 K b_c (where b_c is the Lorentz half-linewidth at half absorption height per atmosphere), the temperature dependence of the air-broadened halfwidth n , the atmospheric temperature T and the atmospheric pressure P . The results of all the error studies are summarized in Table 2. For the Jungfraujoch data, the root of the sum of the squares (r.s.s.) of the individual uncertainty values is 7% or 0.08 p.p.m.v. The r.s.s. for the Kitt Peak spectrum is 6% or 0.09 p.p.m.v. These values have been adopted as the best estimate of the total experimental error. However, note that valid error estimates are difficult to determine for spectroscopic studies because of the difficulty in quantifying the errors for the many parameters involved in the analysis. The agreement between the Kitt Peak value and gas chromatograph determinations for the same epoch and nearly the same latitude^{6–8} suggests that we may have overestimated the total experimental error.

The calculations presented in Table 2 indicate that systematic errors in the line parameters produce about the same relative error in CH_4 concentration for both the Jungfraujoch and Kitt Peak spectra. Therefore, the average rate of increase in CH_4 has been determined more accurately than indicated by the total uncertainty limits assigned to both values. Assuming that errors in determining the rate of increase in CH_4 concentration result only from uncorrelated errors from sources c–h in Table 2, we calculate the average rate of increase of CH_4 concentration in the troposphere to be $1.1 \pm 0.2\%$ yr^{-1} from 1951 to 1981.

Comparison with previous analyses

It is important to note that the CH_4 column amount determined from our analysis is 71% of that inferred by Nielsen and Migeotte³⁷ from measurements of the $2\nu_3$ band in another set of 1951 Jungfraujoch solar spectra. In this earlier work, a curve of growth analysis method and the laboratory measurements of Goldberg *et al.*³⁸ were used to derive the total vertical column amount and the effective rotational temperature of CH_4 above the Jungfraujoch station. Although some of the discrepancy no doubt results from the use of the two different methods of analysis, we believe that most of the discrepancy is caused by the assumption of the Goldberg *et al.* intensities³⁸, which are considerably lower than several more recent studies.

Table 3 compares the Goldberg *et al.* intensities³⁸ with newer measurements^{39–42} for the R0 and R5 lines used in Nielsen and

Table 3 Comparison of intensities reported for the R0 and R5 lines of the $2\nu_3$ band of $^{12}\text{CH}_4$

Ref.	Intensity ($\text{cm}^{-2} \text{atm}^{-1}$ at 296 K)	
	R0	R5
38	0.0098	0.0600
39	0.0095	
40	0.0134	0.0734
41	0.0166	0.0827
42	0.0128	

Migeotte's analysis³⁷. (The values have been converted to a standard set of intensity units to facilitate comparison.) The R10 line was also used by Nielsen and Migeotte³⁷ but, to our knowledge, there have been no more recent determinations of its intensity. Except for Fink *et al.*³⁹ results for R0, the newer intensity values are all considerably larger than those reported by Goldberg *et al.*³⁸, but there is considerable scatter in the measurements. A higher intensity for R0 of $0.0143 \text{ cm}^{-2} \text{ atm}^{-1}$ at 298 K has also been derived by J. S. Margolis (personal communication) from a preliminary analysis of new Kitt Peak laboratory spectra of the $2\nu_3$ band. As an additional check, we have analysed the R0 line in a Kitt Peak solar spectrum recorded with a zenith angle of 49.37° on 10 November 1981. To obtain a CH_4 tropospheric mixing ratio of 1.60 p.p.m.v., an intensity value of $0.0147 \text{ cm}^{-2} \text{ atm}^{-1}$ at 296 K is required, again considerably larger than the Goldberg *et al.*³⁸ intensity. If the Fink *et al.*³⁹ results are excluded, the average of the newer laboratory determinations is 1.40 times larger than the Goldberg *et al.*³⁸ values. Simply scaling the Nielsen and Migeotte³⁷ column amounts by the inverse of this factor produces a CH_4 vertical column amount of 1.49×10^{19} molecules cm^{-2} , corresponding to a CH_4 tropospheric mixing ratio of 1.14 p.p.m.v. The same intensities³⁸ were used by Goldberg⁴³ to analyse infrared solar spectra recorded from Lake Angelus, Michigan (elevation 296 m), and from Mt Wilson, California (elevation 1,750 m), in 1949–1950. If the same scaling factor for the intensity error is adopted along with the case B profile shape, we determine values of 2.28×10^{19} molecules cm^{-2} and 1.17 p.p.m.v. and 1.85×10^{19} molecules cm^{-2} and 1.15 p.p.m.v. for the CH_4 vertical column amount and tropospheric mixing ratio above Lake Angelus and Mt Wilson, respectively. These revised values for the ~1950 CH_4 tropospheric mixing ratio are in excellent agreement with the result of our detailed analysis of the Jungfraujoch spectra, but they should be regarded as subject to a large uncertainty because of the scatter in the intensity values reported for the $2\nu_3$ band. It is interesting to note that a gas chromatographic analysis of air in the troposphere in 1951 indicated a CH_4 mixing ratio of between 1.1 and 1.2 p.p.m.v.^{44,45}, in excellent agreement with our results.

Conclusions

The major finding reported in this paper, that historic solar spectra indicate a significantly lower concentration of CH_4 (1.14 p.p.m.v.) in 1951, has important implications for the fol-

lowing reasons: (1) the result extends the historic baseline of atmospheric measurements of CH_4 ; (2) the results reported here are consistent with a global increase of atmospheric CH_4 of $1.1 \pm 0.2\% \text{ yr}^{-1}$ from 1951 to 1981, assuming a constant rate of increase over this period. This suggests at least a 30-yr increase in atmospheric CH_4 , a significantly longer time increase than previously reported, based on atmospheric measurements. It is interesting to note that the average CH_4 increase of $1.1 \pm 0.2\% \text{ yr}^{-1}$ from 1951 to 1981 reported here is comparable with the annual CH_4 increase deduced from several years of continuous gas chromatograph measurements in the late 1970s and early 1980s; (3) the earlier reported higher values of CH_4 deduced from ground-based infrared solar spectra^{37,43} can be significantly lowered using improved spectroscopic parameters; (4) the atmospheric lifetime of CH_4 is long (~7 yr) compared with the 1–2 yr required to mix a species uniformly throughout both hemispheres⁴⁶. Hence, a single measurement of CH_4 , as reported here, is characteristic of its global concentration. Therefore, our measurement has important implications for the global budget of CH_4 . The 30-yr increase of methane reported here may be caused by an increase in the global production rate of CH_4 (which includes anaerobic decomposition of organic matter, enteric fermentation in ruminants, biomass burning and natural gas leakage), a decrease in the global destruction rate of CH_4 (which is controlled by the reaction of CH_4 with the hydroxyl radical OH), or a combination of both^{47,48}. The implications of the Jungfraujoch spectra-deduced concentration of CH_4 and other trace radiative-active species and their impact on global species budgets and on the photochemistry of the atmosphere will be discussed in an accompanying paper⁴⁹.

We thank D. J. Richardson for help with the digitizing of the Jungfraujoch solar spectrum and S. A. Motley for assistance in locating a copy of the Jungfraujoch solar atlas and the many references used in this study. We also thank J. W. Brault and G. M. Stokes for the Kitt Peak solar spectra and P. Hachler for communicating the Swiss meteorological data for 1951; R. K. Seals Jr, J. M. Russell III and J. D. Lawrence Jr for useful discussions and encouragement; and S. A. Penkett for pointing out the analysis of the chemical composition of tropospheric air performed by E. Gleuckauf at Harwell in the early 1950s. The interferometer in the NSO on Kitt Peak is operated by the Association of Universities for Research in Astronomy, Inc., under contract with the NSF. T.M. is a NASA-MRC Research Associate.

Received 28 March; accepted 25 July 1985.

- Levy, H. *Planet Space Sci.* **20**, 919–935 (1972).
- Crutzen, P. J. in *Physics and Chemistry of the Upper Atmosphere* (ed. McCormac, B. M.) 110–124 (Reidel, Dordrecht, 1973).
- Wang, W. C., Yung, Y. L., Lacy, A. A., Mo, T. & Hansen, J. E. *Science* **194**, 685–690 (1976).
- Graedel, T. E. & McRae, J. E. *Geophys. Res. Lett.* **7**, 977–979 (1980).
- Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **86**, 9826–9832 (1981).
- Seiler, W. in *Chemistry of the Unpolluted and Polluted Troposphere* (NATO Advanced Study Institute, Corfu, 1981).
- Blake, D. R. *et al. Geophys. Res. Lett.* **9**, 477–480 (1982).
- Khalil, M. A. K. & Rasmussen, R. A. *J. geophys. Res.* **88**, 5131–5144 (1983).
- Fraser, P. J., Khalil, M. A. K., Rasmussen, R. A. & Steele, L. P. *J. atmos. Chem.* **1**, 125–135 (1984).
- Ehaldi, D. H., Zander, R. J. & Lamontagne, R. A. *J. geophys. Res.* **88**, 8442–8446 (1983).
- Robbins, R. C., Cavanagh, L. A., Salas, L. J. & Robinson, E. *J. geophys. Res.* **78**, 5341–5344 (1973).
- Craig, H. & Chou, C. C. *Geophys. Res. Lett.* **9**, 1221–1224 (1982).
- Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **89**, 11599–11605 (1984).
- Migeotte, M., Neven, L. & Swensson, J. *Mém. Soc. r. Sci. Liège Spec. Vol. 1* (1956).
- Migeotte, M. *Mém. Soc. r. Sci. Liège 1st Ser.* **1**, 525–634 (1945).
- Migeotte, M. & Neven, L. *Mém. Soc. r. Sci. Liège* **12**, 165–178 (1952).
- Rinsland, C. P. *et al. NASA Tech. Mem.* 85764 (1984).
- Rothman, L. S. *et al. Appl. Opt.* **22**, 2247–2256 (1983).
- Labitzke, K. *et al. Climatology of the Stratosphere in the Northern Hemisphere Pt 1* (Institut für Meteorologie der Freien Universität, Berlin, 1972).
- Barnett, J. J. & Corney, M. Draft of a New Reference Atmosphere for the Middle Atmosphere, COSPAR/SCOSTEP Task Group on Reference Middle Atmospheres MAP Handbook Vol. 16 (in preparation).
- Houghton, J. T. *The Physics of Atmospheres* (Cambridge University Press, 1977).
- Gallery, W. O., Kneizys, F. X. & Clough, S. A. *Envir. Res. Pap.* **828** (AFGL-TR-83-0065, 1983).
- Jones, R. L. & Pyle, J. A. *J. geophys. Res.* **89**, 5263–5279 (1984).
- McClatchey, R. A., Fenn, R. W., Selby, J. E. A., Volz, F. E. & Garing, J. S. *Envir. Res. Pap.* **411** (AFCL-72-0497, 1972).
- Camy-Peyret, C., Flaud, J.-M., Laurent, J. & Stokes, G. M. *Geophys. Res. Lett.* **10**, 35–38 (1983).
- Flaud, J.-M., Camy-Peyret, C., Cariolle, D., Laurent, J. & Stokes, G. M. *Geophys. Res. Lett.* **10**, 1104–1107 (1983).
- Smith, M. A. H. *NASA Tech. Mem.* 83289 (1982).
- Rothman, L. S. *et al. Appl. Opt.* **22**, 1616–1627 (1983).
- McMahon, J., Troup, G. J., Hubbert, G. & Kyle, T. G. *J. quant. Spectrosc. Rad. Transf.* **12**, 797–805 (1972).
- Varanasi, P. J. *quant. Spectrosc. Rad. Transf.* **15**, 281 (1975).
- Malathy Devi, V., Fridovich, B., Jones, G. D. & Snyder, D. G. S. *J. molec. Spectrosc.* **97**, 333–342 (1983).
- Malathy Devi, V., Fridovich, B., Jones, G. D., Snyder, D. G. S. & Neuendorffer, A. *Appl. Opt.* **21**, 1537–1538 (1982).
- Rinsland, C. P. *et al. J. geophys. Res.* **87**, 11119–11125 (1982).
- Rinsland, C. P., Boughner, R. E., Larsen, J. C., Stokes, G. M. & Brault, J. M. *J. geophys. Res.* **89**, 9613–9622 (1984).
- Kostkowski, H. J. & Bass, A. M. *J. opt. Soc. Am.* **46**, 1060–1064 (1956).
- Rinsland, C. P. *et al. J. quant. Spectrosc. Rad. Transf.* **30**, 327–334 (1983).
- Nielsen, A. H. & Migeotte, M. *Ann. Astrophys.* **15**, 134–141 (1952).
- Goldberg, L., Mohler, O. C. & Donovan, R. E. *J. opt. Soc. Am.* **42**, 1–6 (1952).
- Fink, U., Rank, D. H. & Wiggins, T. A. *J. opt. Soc. Am.* **54**, 472–474 (1964).
- Margolis, J. S. *J. quant. Spectrosc. Rad. Transf.* **13**, 1097–1103 (1973).
- Sarangi, S. & Varanasi, P. J. *quant. Spectrosc. Rad. Transf.* **14**, 365–376 (1974).
- Fox, K., Halsey, G. W., Daunt, S. J., Blass, W. E. & Jennings, D. E. *J. chem. Phys.* **72**, 4657–4660 (1980).
- Goldberg, L. *Astrophys. J.* **113**, 567–582 (1951).
- Gleuckauf, E. in *Compendium of Meteorology* (ed. Malone, T. F.) (Am. met. Soc., 1951).
- Penkett, S. A. in *Atmospheric Chemistry* (ed. Goldberg, E. D.) 329–355 (Springer, Berlin, 1982).
- Chameides, W. L. & Davis, D. D. *Chem. Engng News* **60**, 38–52 (1982).
- Levine, J. S. & Allario, F. *Envir. Monitor. Assess.* **1**, 263–306 (1982).
- Levine, J. S. *Satellite Sensing of a Cloudy Atmosphere: Observing the Third Planet* (ed. Henderson-Sellers, A.) (Taylor and Francis, London, 1984).
- Levine, J. S., Rinsland, C. P. & Tennille, G. M. *Nature* **318**, 254–257 (1985).

Free tropospheric carbon monoxide concentrations in 1950 and 1951 deduced from infrared total column amount measurements

Curtis P. Rinsland & Joel S. Levine

Atmospheric Sciences Division, NASA Langley Research Center, Hampton, Virginia 23665, USA

Published total vertical column amounts of carbon monoxide deduced from infrared solar spectra recorded during 1950–51 in the Swiss Alps have been reanalysed based on improved spectroscopic parameters for CO and a curve of growth. From a comparison of the 1950–51 results and modern measurements, an average increase of ~2% per year in the free tropospheric concentration of CO above Europe is estimated for 1950–77.

CARBON monoxide (CO) was discovered by Migeotte in the Earth's atmosphere by its absorption signature in infrared, ground-based spectra of the Sun¹. (Migeotte² also discovered methane (CH₄) using the same technique.) Carbon monoxide is an important species in the photochemistry of the troposphere. The reaction between CO and the hydroxyl radical (OH), the dominant oxidizing species in the troposphere, controls the global destruction of both of these species. The reaction between these two gases accounts for more than 80% of the global destruction of OH and the total destruction of CO, and the reaction between CO and OH which produces an atom of hydrogen (H) may also lead to the photochemical production of ozone (O₃) in the troposphere³.

Measurements indicate that at ground level, the mixing ratio of CO is between 120 and 200 parts per 10⁹ by volume (p.p.b.v.) in the Northern Hemisphere compared with 40–60 p.p.b.v. in the Southern Hemisphere³. The surface mixing ratio of CO is higher than in the free troposphere, at least for the Northern Hemisphere³. Measurements in the Northern Hemisphere indicate the background level of CO varies by about a factor of two with season^{4,5}, with a seasonal maximum in CO of about 150 p.p.b.v. in early spring and a seasonal minimum of about 70 p.p.b.v. in late summer at Cape Meares, Oregon, a remote site at 45° N (ref. 4).

There have been several recent studies on the possible temporal change of CO mixing ratios in the troposphere. Khalil and Rasmussen⁴ found a long-term increase of about 6% yr⁻¹ based on 44 months of continuous Cape Meares data (1979–82). Analyses of two other CO data sets by the same authors also indicate a long-term increase in tropospheric CO. The first of these two other CO data sets is unpublished and was obtained at Cape Grim, Tasmania (42° S) between October 1981 and April 1984. These results suggest that CO may be increasing by about 4% yr⁻¹ in the Southern Hemisphere. However, we note that Seiler *et al.*⁷ have compared Southern Hemispheric CO mixing ratios measured in 1971–72 with CO mixing ratios measured continuously at Cape Point (34° S) between 1978 and 1981 and conclude that Southern Hemisphere CO mixing ratios could not have changed by more than 5–10% over the past decade. Measurements of the total column amount of CO have been deduced from infrared spectroscopic measurements recorded between 1970 and 1982 (excluding a gap in the measurements between 1977 and 1980) in the Soviet Union^{8–11}. The reanalysis by Khalil and Rasmussen⁶ of the data reported by Dvoryashina *et al.*⁸ suggests that CO may have increased by 2% (±1%) yr⁻¹ since 1974, but there is no evidence for an increase in CO prior to 1974. The long-term increase of tropospheric CO suggested by some of these studies^{4,6} is very important and may indicate a significant anthropogenic perturbation to tropospheric composition and/or chemistry. Of equal importance is the conclusion that the long-term CO increase is not apparent in the measurement records in the Soviet Union prior to 1974.

As emphasized in these studies^{4,6}, it is very difficult to separate

the relatively small systematic long-term trend in background CO concentration from the large seasonal cycle and fluctuations in concentration caused by seasonal variations in CO production, destruction, and transport. For this reason, it is desirable to have continuous measurements from a remote site spanning several decades. Although no such database exists, we believe that important information on the long-term trend in CO concentration can be deduced from analysis of historic solar spectra. Since atmospheric concentrations of CO are difficult to store in containers¹², historic solar spectra may represent the only record of early CO concentrations. A number of infrared solar spectra obtained in the early 1950s show prominent telluric lines of CO and have been analysed for total CO column amounts^{13–16}. With the exception of the study by Benesch *et al.*¹³, these spectra were recorded near large urban areas, which are significant sources of CO.

The Benesch *et al.*¹³ measurements are unique in that they were obtained from spectra recorded at a remote, clean-air, high-altitude observatory in the Swiss Alps. We have previously analysed spectra obtained more than three decades ago at the same site to deduce the tropospheric CH₄ level¹⁷. In this article, we reanalyse the early CO column amount measurements of Benesch *et al.*¹³ using modern spectroscopic parameters and obtain estimates of the free tropospheric volume mixing ratio of CO above Europe on 9 days in 1950–51.

Measurements and analysis

The solar spectra analyzed by Benesch *et al.*¹³ were recorded at the Jungfraujoch Scientific Station in the Swiss Alps (46.5° N, 8.0° E, altitude 3,578 m) on three days in August 1950 and on six days between April and September 1951. The observations were recorded with a Pfund-type prism-grating spectrometer and with a Perkin-Elmer thermocouple as a detector. The instrumentation has been described in detail^{18,19} and a number of the spectra have been published in a solar atlas²⁰, including measurements which show prominent CO absorption lines on 25 April 1951 and 7 June 1951. Most of the spectra analyzed by Benesch *et al.*¹³ are unpublished.

Benesch *et al.*¹³ determined total vertical column amounts of CO (in units of cm-atm at STP) from measurements of the equivalent width of the R3 line of the (1-0) vibration-rotation band of ¹²C¹⁶O at 2,158,300 cm⁻¹. The column amounts were derived assuming that the absorption of the R3 line in the solar spectra occurs in the square root region of the curve of growth. In the square root region, the equivalent width *W* is given by^{15,21}

$$W = 2(Sb_L Lu)^{1/2} \quad (1)$$

where *b_L* is the Lorentz half-linewidth at absorption half height, *S* is the line intensity per molecule, *L* is Loschmidt's number, and *u* is the absorber column amount in units of cm-atm at STP. Equation (1) assumes the equivalent width has been obtained from an integration of the absorption over all frequen-

Table 1 Comparison of line parameters at 296 K for the R3 line of the (1-0) band of $^{12}\text{C}^{16}\text{O}$

Parameter	Units	Benesch <i>et al.</i> ¹³ Value	Present study	
			Value	Ref.
S	cm^{-1} molecule	2.91×10^{-19}	3.47×10^{-19}	Chackerian <i>et al.</i> ²²
b_L^0 (CO-air)	$\text{cm}^{-1} \text{ atm}^{-1}$	0.046	0.0672	Nakazawa & Tanaka ²⁴
n		0.0	0.75	Varanasi ²⁶

cies. The halfwidth b_L is related to the halfwidth per atmosphere b_L^0 at reference temperature T_0 by

$$b_L = b_L^0 \left(\frac{T}{T_0} \right)^{-n} p \quad (2)$$

where T is the temperature in K, p is the pressure in atm, and n is the coefficient of the temperature dependence of the halfwidth. Benesch *et al.*¹³ assumed the air-broadened halfwidth to be independent of temperature ($n = 0$).

A recent comprehensive survey of the literature shows a large number of measurements of intensities and collisional broadening parameters for the 1-0 band of $^{12}\text{C}^{16}\text{O}$ (ref. 21). From these results, we have attempted to select the most accurate parameters for the R3 line. The adopted values converted to the standard reference temperature of 296 K are listed in Table 1 along with those assumed in the Benesch *et al.*¹³ analysis. The $^{12}\text{C}^{16}\text{O}$ line intensities of Chackerian *et al.*²² have a quoted absolute uncertainty of $\pm 3\%$. We multiplied the Chackerian *et al.*²² intensity for R3 by 0.98652 to account for the concentration of $^{12}\text{C}^{16}\text{O}$ in natural CO (ref. 23). The air-broadened halfwidth b_L^0 (CO-air) assumed in this study was calculated from the experimental halfwidths of Nakazawa and Tanaka²⁴ assuming the relation

$$b_L^0(\text{CO-air}) = 0.79 b_L^0(\text{CO-N}_2) + 0.21 b_L^0(\text{CO-O}_2) \quad (3)$$

where $b_L^0(\text{CO-N}_2)$ and $b_L^0(\text{CO-O}_2)$ are the experimental N_2 - and O_2 -broadened halfwidths, respectively. We note that the Varghese and Hanson²⁵ N_2 -broadened halfwidth for R3 agrees within 4% with the Nakazawa and Tanaka value²⁴ and that the calculated air-broadened halfwidth agrees closely with the value of $0.0695 \pm 0.0036 \text{ cm}^{-1} \text{ atm}^{-1}$ at 300 K determined for air-broadening by Varanasi²⁶.

Benesch *et al.*¹³ assumed an effective temperature of 230 K and an effective pressure of 0.3 atm for the CO molecules above the Jungfraujoch station. If these values are also adopted as a first approximation, the column amounts u_B derived by Benesch *et al.*¹³ can be compared to column amounts u_R determined with the revised spectroscopic parameters. Substituting the values from Table 1 into equations (1) and (2), we find for a given measured equivalent width that

$$\frac{u_R}{u_B} = 0.475 \quad (4)$$

Hence, by assuming values that are too low for both the R3 intensity and halfwidth, Benesch *et al.*¹³ overestimated the CO column amount above the Jungfraujoch station by about a factor of 2.

More accurate corrections to the Benesch *et al.*¹³ values have been obtained from a curve of growth derived from equivalent widths measured from synthetic spectra calculated with a 17-layer atmospheric model. Table 2 presents the pressure-temperature and CO reference mixing ratio profile assumed in the analysis. The pressures and temperatures are density-weighted values calculated from the 1976 US Standard Atmosphere²⁷. The reference CO mixing ratio profile adopted in the analysis is based on midlatitude Northern Hemisphere measurements. The stratospheric portion of this profile is taken from a set of reference gas concentration profiles²⁸ and is based on the measurements of Fabian *et al.*²⁹ and Ehhalt and Tönnissen³⁰. Below 11 km, a constant mixing ratio of 130 p.p.b.v. has been assumed based on an approximate average of free tropospheric measurements over Europe, although the actual measurements exhibit a wide scatter around the average value, as shown in

Fig. 10 of Logan *et al.*³. A Voigt profile has been assumed for the CO line shape.

Our spectral simulations indicate that the R3 CO line is slightly blended with a feature due to N_2O and solar CO on the low wavenumber wing and a feature due to N_2O on the high wavenumber wing. Also, the R3 line contains a contribution due to the absorption by the same transition in the solar atmosphere. Because the blends on both wings of the line were marked in the solar atlas²⁰ and identified in the list of measures³¹, we have assumed that Benesch *et al.*¹³ corrected their measured R3 line equivalent widths for both telluric and solar line absorption by these blending transitions. Therefore, only telluric absorption by the R3 line was included in our curve of growth calculations. The revised parameters in Table 1 were assumed along with the position and lower state energy from the 1982 Air Force Geophysics Laboratory line parameters compilation³².

The CO volume mixing ratio profile in Table 2 corresponds to a vertical column amount of 0.0512 cm-atm STP. To determine the curve of growth, a sequence of 10 synthetic spectra were calculated by scaling this mixing ratio distribution by multiplicative factors ranging from 0.1 to 2.4. Each spectrum has been convolved with a gaussian instrument function having a width corresponding to the resolution of the Jungfraujoch measurements as given in Table 1 of the solar atlas²⁰. Because of significant pressure broadening in the troposphere, absorption in the line wings is important in determining the total equivalent width. Since Benesch *et al.*¹³ did not discuss corrections to their equivalent widths for the contribution from the line wings, we assume their values were derived from integrating over only a finite wavenumber interval. From examination of the published solar spectra in this region^{13,20} and our calculated synthetic spectra, we estimated their integration limits to have been $2,157.5$ – $2,159.1 \text{ cm}^{-1}$. Outside of this interval, absorption by the R3 line changes slowly with wavenumber and is overlapped by absorption from other lines in the Jungfraujoch solar spectra. We adopted these integration limits in analyzing the synthetic spectra and determined the background (measured 100% transmittance level) from a least-squares fit to data within 0.1 cm^{-1} of the integration limits. The derived equivalent widths are fairly insensitive to the adopted integration limits. For example, if the

Table 2 Atmospheric parameters assumed in the analysis

Layer boundaries (km)	Temperature (K)	Pressure (mbar)	CO mixing ratio (p.p.b.v.)
3.578–5.0	260.4	595.3	130
5.0–7.5	247.9	461.2	130
7.5–9.0	234.7	345.1	130
9.0–11.0	224.2	267.3	130
11.0–14.0	217.3	184.2	72.5
14.0–17.0	216.6	115.1	35.0
17.0–20.0	216.6	71.9	18.8
20.0–22.0	217.6	47.9	15.5
22.0–24.0	219.5	35.1	16.5
24.0–26.0	221.5	25.8	17.5
26.0–28.0	223.5	19.1	18.5
28.0–30.0	225.5	14.1	19.5
30.0–32.0	228.4	10.4	21.0
32.0–35.0	233.3	7.35	23.5
35.0–40.0	242.5	4.33	29.2
40.0–60.0	260.0	1.55	30.0
60.0–100.0	230.7	0.11	30.0

integration range is decreased to 2,157.6–2,159.0 cm^{-1} , the equivalent widths determined from the synthetic spectra decrease by 0.8–2.1%. An increase in the integration range to 2,157.4–2,159.2 cm^{-1} increases the calculated equivalent widths by 0.6–1.7%. As expected, the largest changes in equivalent width were found for the calculations with the largest CO profile multiplicative scaling factor.

For each of the 11 measurements reported by Benesch *et al.*¹³, a revised total vertical column amount and a corresponding free tropospheric volume mixing ratio of CO were derived from the curve of growth using the following steps:

- (1) The solar zenith angle was estimated from the date and approximate time of observation (listed in Fig. 5 of Benesch *et al.*¹³).
- (2) The CO column amount was calculated from the product of the secant of the solar zenith angle and the reported total vertical column amount.
- (3) From this result and the line parameters assumed by Benesch *et al.*¹³, the measured equivalent width was estimated using equation (1).
- (4) A revised CO column amount was derived from this equivalent width using the curve of growth.
- (5) The revised vertical column of CO was calculated by dividing the revised CO column amount by the secant of the solar zenith angle. The corresponding volume mixing ratio below 11 km is assumed to be the free tropospheric mixing ratio of CO for that date and time.

The revised total vertical column amounts and corresponding free tropospheric volume mixing ratios of CO are given in Table 3. For comparison, the total vertical column amounts of CO deduced by Benesch *et al.*¹³ are also listed. On the average, the revised values are 59% of the Benesch *et al.*¹³ column amounts. This result is slightly higher than the 47.5% estimate obtained from the simple scaling procedures discussed previously.

As a check on our curve of growth results, the region in the Jungfraujoch solar atlas²⁰ containing the R3 line of CO was analysed using the revised set of CO line parameters (Table 1) and the technique of nonlinear least squares spectral curve fitting. These spectral data were recorded at about 6:30 UT on April 25, 1951, corresponding to a solar zenith angle of 69.3° (measurement no. 5 in Table 3). Solar CO lines in the region were included in the fitting procedure as described by Rinsland *et al.*³³; otherwise, the analysis method was the same as that used to determine the 1951 CH_4 tropospheric mixing ratio from the Jungfraujoch solar spectra¹⁷. The fitting results are shown in Fig. 1. The free tropospheric mixing ratio of CO deduced from this spectral analysis is 99 p.p.b.v., somewhat lower than the value of 110 p.p.b.v. obtained from the curve of growth analysis for the same measurements.

It is also important to be sure that the mixing ratio results do not depend on the line selected for the analysis. Therefore, the spectral fitting procedure and a similarly revised set of CO line parameters have been applied to the analysis of the spectral

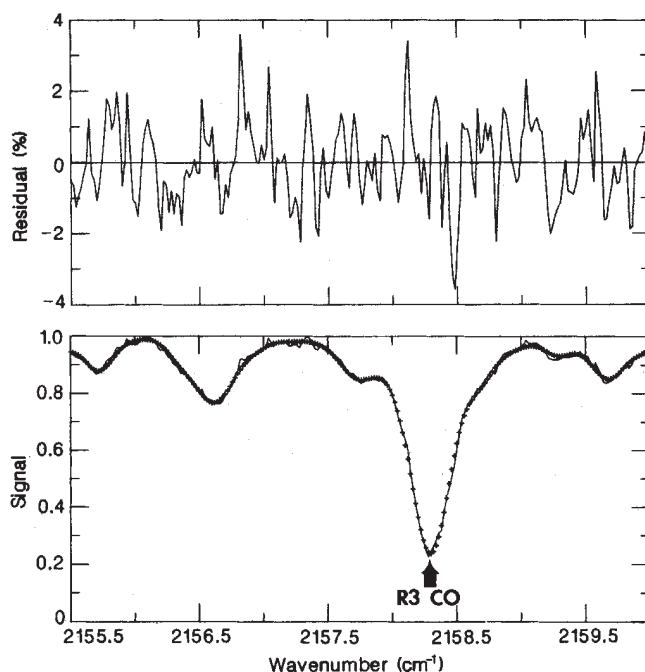


Fig. 1 Comparison between a solar absorption spectrum recorded at the Jungfraujoch Scientific Station on 25 April 1951 (solid line) and least-squares best-fit to the data (crosses). The measured spectrum was obtained about 6:30 UT at a solar zenith angle of 69.3° and has been normalized to the largest measured intensity in the fitted region. The residuals (observed minus calculated) are plotted at top.

interval containing the P14 line at 2,086.322 cm^{-1} and the P15 line at 2,082.002 cm^{-1} . These data were also recorded on 25 April 1951. The value of 103 p.p.b.v. determined from this analysis is in good agreement with the values obtained from the spectral fitting and curve of growth analyses of the R3 line (cited above).

The CO volume mixing ratios in Table 3 range from 30 to 110 p.p.b.v., a factor of 3.7 to 1, indicating large variability in CO abundance (the extreme limit of 4.4 to 1 deduced by Benesch *et al.*¹³ is reduced slightly by curve-of-growth effects). Although most of this variability is probably real, some of the scatter in the measurements is likely to be due to changes in the altitude of the tropopause (a constant value of 11 km has been assumed), errors in the equivalent width measurements of Benesch *et al.*¹³, and errors resulting from the assumption of a standard atmosphere pressure-temperature profile in the analysis. It is interesting to note that Benesch *et al.*¹³ observed significant changes in the CO total vertical amount on time scales of several hours. They were unable to find a meaningful correlation between these variations and meteorological data.

The CO mixing ratios observed on the 3 days of measurement in August 1950 are lower than those observed during August and September 1951 and much lower than all modern measurements of free tropospheric CO near the same latitude. Therefore, we investigated the available meteorological records for unusual conditions, for example very low tropopause altitude or intrusion of stratospheric air during a tropopause folding event. Surface weather maps for the 3 days of observation (J. Tierney, personal communication) show a weak anti-cyclonic circulation pattern near Switzerland with no evidence for the vigorous tropospheric depressions and associated fronts typically observed during a significant intrusion of stratospheric air into the troposphere³⁴. Radiosonde measurements from Payerne (46.5° N, 6.6° E) for 05 h U.T. indicate tropopause altitudes of 10.5 km for 19 August, 13.0 km for 21 August and 12.3 km for 30 August (no radiosonde measurements were made on 20 August, P. Hachler, personal communication), close to the nominal tropopause height of 11 km assumed in our calculations for all dates. However, the conventional temperature lapse rate

Table 3 Total vertical column amount u_{CO} (in cm-atm at STP) and corresponding free tropospheric mixing ratio β_{CO} of carbon monoxide above the Jungfraujoch Scientific Station in 1950–51

Measurement no.	Date	Time (UT)	Benesch <i>et al.</i> ¹³	This study	
			u_{CO}	u_{CO}	β_{CO}
1	19 August 1950	12:00	0.029	0.018	46
2	20 August 1950	9:00	0.017	0.012	30
3	30 August 1950	8:30	0.020	0.013	32
4	24 April 1951	9:30	0.057	0.032	82
5	25 April 1951	6:30	0.075	0.043	110
6	7 June 1951	7:30	0.058	0.032	82
7	7 June 1951	13:00	0.068	0.038	98
8	14 August 1951	9:00	0.048	0.027	70
9	20 September 1951	8:30	0.068	0.038	96
10	20 September 1951	13:00	0.042	0.024	61
11	21 September 1951	16:00	0.043	0.024	62

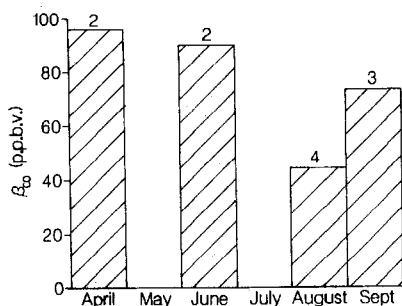


Fig. 2 Monthly averaged free tropospheric volume mixing ratios of CO deduced from the 1950–51 Jungfraujoch solar spectra. The number of measurements is shown for each month.

criterion used to define the tropopause altitude may be a poor indicator of the separation of stratospheric and tropospheric air during a major tropopause folding event³⁴. Therefore, potential temperature versus log. pressure plots were constructed, and these do not show the large low-altitude vertical gradients of isentropes characteristic of a tropopause fold³⁴. On the basis of the available evidence we have retained these three measurements in our analysis of the long-term trend, and we find it quite interesting that such very low CO mixing ratios apparently existed in the free troposphere above Europe in 1950.

In Fig. 2, all of the measurements within a given month have been averaged to evaluate the seasonal trend in CO. As can be seen, despite the limited number of measurements, there is some evidence for a seasonal cycle in the 1950–51 CO mixing ratios, with higher values in spring than in late summer and early fall, in rough agreement with modern studies^{4–6}. Unfortunately, the number of measurements and their distribution with season are insufficient and the scatter of the results is too large to define accurately the seasonal cycle of atmospheric CO in 1950 and 1951.

Fortuitously, the Jungfraujoch measurements are distributed at about the times of year when modern Northern Hemisphere measurements^{4,5} indicate the CO concentrations range from their highest values (early spring) to their lowest values (late summer). Therefore, a straight average of the 4-monthly means in Fig. 2 can be used to calculate a seasonally-averaged value with minimal sampling bias. The result is 76 p.p.b.v., a value considerably lower than most published measurements for the free troposphere over Europe (see Fig. 10 of Logan *et al.*³). The estimated uncertainty in the 1950–51 average is rather large (± 25 p.p.b.v.), primarily because of the large inferred variability, the limited number of measurements, uncertainties in the assumed CO vertical mixing ratio distribution (the measurements presented in Fig. 10 of Logan *et al.*³ and the measurements of Seiler and Fishman³⁵ show some evidence for a decrease of CO volume mixing ratio with altitude in the free troposphere), and uncertainties in the revised R3 CO spectroscopic line parameters. The assumptions adopted in the analysis to infer the Benesch *et al.*¹³ equivalent widths also contribute to the uncertainty.

Estimate of long-term (CO) trend

The most accurate estimate of the long-term trend in tropospheric CO concentration would result from comparison of the 1950–51 values with values deduced in the same way from modern Jungfraujoch solar spectra. This procedure would reduce potential systematic errors resulting from uncertainties in the assumed values for the spectroscopic line parameters and the CO vertical mixing ratio distribution and from potential systematic errors resulting from the method of analysis. Unfortunately, no modern Jungfraujoch spectra covering the CO region have been published. Therefore, we have been forced to assume modern CO measurement values from the literature.

Figure 10 of Logan *et al.*³ presents a number of free tropospheric measurements of CO obtained over Europe by Seiler *et al.*³⁶ in November and December 1976 (50–60° N) and by Fabian

*et al.*³⁷ in June and September 1977 (44° N). Near 6 km, the mean altitude of tropospheric CO molecules above the Jungfraujoch station (assuming the reference CO vertical distribution in Table 2), these measurements range from 75 to 175 p.p.b.v. with an approximate average value of 130 p.p.b.v. Measurements^{35,38,39} at other longitudes between 1971 and 1978 show similar average free tropospheric CO mixing ratios near 45° N (see Fig. 10 of Seiler and Fishman³⁵). Assuming an average of 130 p.p.b.v. for 1976–77 and our seasonally averaged mixing ratio of 76 p.p.b.v. for 1950–51, an average rate of CO increase of 2.1% yr⁻¹ is obtained for this 27-year period. Uncertainty in the average rate of increase results from uncertainties in the adopted average values for both 1950–51 and 1976–77. Assuming average values ranging from 115 to 145 p.p.b.v. for the latter period and average values for 1950–51 corresponding to the estimated uncertainty limits, the average rate of CO concentration increase for 1950–77 is calculated from the extremes of the uncertainty limits to be between 0.5–4.1% yr⁻¹.

Conclusions

The major conclusion of this study is that the seasonally averaged free tropospheric mixing ratio of CO above Europe in 1950–51 was 76 ± 25 p.p.b.v., a value considerably lower than most modern measurements for the same location. The deduced average rate of increase in free tropospheric CO above Europe is $\sim 2\%$ yr⁻¹ for 1950–77 and is consistent with more recent measurements of increasing CO concentration at sites in both the Northern and Southern Hemispheres^{4,6}. The primary strength of the present analysis is that the average rate of CO increase has been estimated from measurements separated by 27 years, over which time the small rate of increase in CO concentration has accumulated to a value which allows it to be separated fairly well from short-term fluctuations and the seasonal cycle of CO. Nonetheless, the accuracy in the estimated average rate of increase in free tropospheric CO over Europe is limited considerably by the large variability of CO, as deduced both from our analysis of the limited set of 11 measurements from 1950–51 and from more numerous recent measurements. Because of the large spatial as well as temporal variability of tropospheric CO, the globally averaged rate of change in CO concentration cannot be inferred from our results. However, when the published measurements of increasing CO at both Northern and Southern Hemisphere sites^{4,6} are considered in addition to our results, we believe there now exists considerable experimental evidence for a global increase in tropospheric CO concentration. Some implications of the Jungfraujoch spectra-deduced increases in tropospheric CO and CH₄ are discussed in the accompanying paper.⁴⁰

H. G. Reichle Jr and S. A. Motley helped us locate many of the pertinent papers on tropospheric CO cited in this work. We also thank Thomas Miles for his assistance with the analysis of the August 1950 meteorological data and G. Roland for sending us a copy of the Jungfraujoch infrared solar atlas.

Received 20 May; accepted 16 September 1985.

1. Migeotte, M. V. *Phys. Rev.* **75**, 1108–1109 (1948).
2. Migeotte, M. *Phys. Rev.* **73**, 519–520; *Astrophys. J.* **107**, 400–403 (1948).
3. Logan, J. A., Prather, M. J., Wofsy, S. C. & McElroy, M. B. *J. geophys. Res.* **86**, 7210–7254 (1981).
4. Khalil, M. A. K. & Rasmussen, R. A. *Science* **224**, 54–56 (1984).
5. Seiler, W., Giehl, H. & Ellis, H. *WMO Spec. Envir. Rep. No. 10*, 31–39 (1976).
6. Khalil, M. A. K. & Rasmussen, R. A. in *Transactions APCA Specialty Conference on the Environmental Impact of Natural Emissions* (ed. Aneja, V. P.) 403 (Air Pollution Control Association, Pittsburgh, 1984).
7. Seiler, W., Giehl, H., Brunke, E. G. & Halliday, E. *Tellus* **36B**, 219–231 (1984).
8. Dvoryashina, E. V., Dianov-Klovov, V. I. & Yurganov, L. N. preprint, USSR Academy of Sciences, Moscow (1982).
9. Dvoryashina, E. V., Dianov-Klovov, V. I. & Yurganov, L. N. *Izv. Akad. Nauk. SSR (Atmos. ocean. Phys.)* **20**, 40–47 (1984).
10. Dianov-Klovov, V. I. & Yurganov, L. N. *Tellus* **33**, 262–273 (1981).
11. Dianov-Klovov, V. I., Fokeyeva, Ye. V. & Yurganov, L. N. *Izv. Akad. Nauk. SSR (Atmos. ocean. Phys.)* **14**, 263–270 (1978).
12. Heidt, L. E. *et al. J. geophys. Res.* **85**, 7329–7336 (1980).
13. Benesch, W., Migeotte, M. & Neven, L. *J. opt. Soc. Am.* **43**, 1119–1123 (1953).
14. Locke, J. L. & Herzberg, L. *Can. J. Phys.* **31**, 504–516 (1953).
15. Shaw, J. H. *Astrophys. J.* **128**, 428–440 (1958).
16. Goldberg, L. in *Handbook of the Solar System* (ed. Kuiper, G. P.) Vol. 2, 479 (University of Chicago Press, 1954).
17. Rinsland, C. P., Levine, J. S. & Miles, T. *Nature* **318**, 245–249 (1985).

18. Migeotte, M. *Mém. soc. roy. sci. Liège*, 1st ser. 1, 525-634 (1945).
19. Migeotte, M. & Neven, L. *Mém. soc. roy. sci. Liège* 12, 165-178 (1952).
20. Migeotte, M., Neven, L. & Swensson, J. *Mém. Soc. roy. sci. Liège, Spec. Vol. 1* (1956).
21. Smith, M. A. H., Rinsland, C. P., Fridovich, B. & Narahari Rao, K. in *Molecular Spectroscopy: Modern Research Vol. 3* (ed. Narahari Rao, K.) 127 (Academic, Orlando, 1985).
22. Chackerian, C. Jr, Guelachvili, G. & Tipping, R. H. *J. quant. Spectrosc. Radiat. Transfer* 30, 107-112 (1983).
23. McClatchey, R. A. et al. Rep. AFCRL-TR-0096, *Environ. Res. Pap. No. 434* (Air Force Cambridge Research Laboratories, Bedford, Massachusetts, 1973).
24. Nakazawa, T. & Tanaka, M. *J. quant. Spectrosc. Radiat. Transfer* 28, 409-416 (1982).
25. Varghese, P. L. & Hanson, R. K. *J. quant. Spectrosc. Radiat. Transfer* 24, 479-489 (1980).
26. Varanasi, P. J. *quant. Spectrosc. Radiat. Transfer* 15, 191-196 (1975).
27. U.S. Standard Atmosphere, 1976. NOAA-S/T 76-1562 (1976).
28. Smith, M. A. H. *NASA tech. Mem.* 83289 (1982).
29. Fabian, P. et al. *J. geophys. Res.* 86, 5179-5184 (1981).
30. Ehhalt, D. H. & Tönnissen, A. *Proc. NATO Advanced Study Institute on Atmospheric Ozone: Its Variations and Human Influences* (ed. Aiken, A. C.) 129-152 (FAA Report No. FAA-EE-80-20, 1980).
31. Migeotte, M., Neven, L. & Swensson, J. *Mém. Soc. roy. sci. Liège, Spec. Vol. 2* (1957).
32. Rothman, L. S. et al. *Appl. Opt.* 22, 2247-2256 (1983).
33. Rinsland, C. P. et al. *Appl. Opt.* 21, 4351-4355 (1982).
34. Danielsen, E. et al. *J. geophys. Res.* 75, 2353-2361 (1970).
35. Seiler, W. & Fishman, J. *J. geophys. Res.* 86, 7255-7265 (1981).
36. Seiler, W., Muller, F. & Oeser, H. *Pure appl. Geophys.* 116, 554-566 (1978).
37. Fabian, P. et al. *J. geophys. Res.* 84, 3149-3154 (1979).
38. Seiler, W. *Tellus* 26, 117-135 (1974).
39. Heidt, L. E. et al. *J. geophys. Res.* 85, 7329-7336 (1980).
40. Levine, J. S., Rinsland, C. P. & Tennille, G. M. *Nature* 318, 254-257 (1985).

The photochemistry of methane and carbon monoxide in the troposphere in 1950 and 1985

Joel S. Levine, Curtis P. Rinsland & Geoffrey M. Tennille*

Atmospheric Sciences Division and *Analysis and Computation Division, NASA Langley Research Center, Hampton, Virginia 23665, USA

The ~1%/year increase in tropospheric methane and ~2%/year increase in tropospheric carbon monoxide deduced from recent analyses of ground-based solar infrared spectra recorded in 1950 and 1951 have very important implications for the photochemistry and chemistry of the troposphere. Photochemical calculations indicate that as a result of the increase of methane and carbon monoxide since 1950-51, levels of the hydroxyl radical, the key species in the photochemistry of the troposphere, may have decreased by about 25%.

RECENT analyses of ground-based solar infrared absorption spectra recorded in 1950 and 1951 at the Jungfraujoch Scientific Station in the Swiss Alps (46.5°N, 3578 m altitude) have led to the discovery that 34-35 years ago, the mean tropospheric mixing ratio of methane (CH₄) was 1.14 ± 0.08 parts per million by volume (p.p.m.v.)¹ or only about 70% of its present day mixing ratio of 1.65 p.p.m.v. and that the average free tropospheric (~5 km) mixing ratio of carbon monoxide (CO) was only about half of its present day mixing ratio at that latitude². Extrapolating the 1951 data point to the present day value suggests that tropospheric CH₄ has increased at an average rate of about $1.1 \pm 0.2\%$ yr⁻¹ over the past 34 years. This rate of increase in CH₄ is comparable to the 1-2% per year increase in tropospheric CH₄ based on nearly continuous ground-based gas chromatograph measurements obtained since about 1977³⁻⁶, and is consistent with CH₄ levels measured in air bubbles embedded in polar ice cores^{7,8}. Extrapolating the 1950-51 data to present day values at the same latitude suggests that tropospheric CO has increased at an average rate of about 2% per year over the past 34-35 years, consistent with very recent continuous ground-based measurements of CO obtained over a 3½ year period (1979-82)⁹.

In this article, we consider the implications of the 1950 and 1951 measurements on the ground production rates of CH₄ and CO and on the tropospheric photochemical coupling between these two gases. At the outset, it must be emphasized that the troposphere is a strongly coupled chemical system of several dozen interacting species controlled by many different processes, including natural and anthropogenic emissions, photochemical and chemical processes, and various heterogeneous loss processes^{10,11}. There are many deficiencies and uncertainties in our understanding of the composition and chemistry of the present-day troposphere. Trying to understand the composition and chemistry of the troposphere more than three decades ago based on measurements of only two species is very difficult at the least. We know nothing about the concentrations of the other several dozen tropospheric species. While our analysis must be considered somewhat speculative, we believe that the analysis and interpretation of historic solar spectra using photochemical

models offer a new and powerful technique to study changes in tropospheric composition and chemistry over a period of several decades.

Photochemical calculations presented here were obtained with a one-dimensional photochemical model of the troposphere¹²⁻¹⁵. The version of the model used in these calculations contains 80 species from the following species families: hydrogen, nitrogen, oxygen, carbon, ammonia, sulphur and halogen (chlorine, bromine and iodine). The non-methane hydrocarbon chemistry^{13,14} was not included in the present calculations to reduce computational costs. The photochemical production of CO resulting from the oxidation of natural and anthropogenic nonmethane hydrocarbons is included in the surface flux boundary condition for CO, as will be described. The calculation of photolysis rates includes the effect of molecular absorption, Rayleigh scattering, Mie scattering, and surface albedo, based on a detailed radiative transfer model¹⁶ which has been incorporated into our photochemical model. The radiative transfer calculation uses optical depths for ozone absorption, Rayleigh scattering, and Mie scattering¹⁷. A major change in the present version of the model is the inclusion of CH₄ as a chemically active species whose vertical profile is calculated using a continuity-transport equation with a surface flux of CH₄ as the lower boundary condition. In earlier versions of the model, the vertical distribution of CH₄, a long-lived gas, was specified as an input parameter, as it is in almost every tropospheric photochemical model. For present day conditions, the following surface boundary conditions were specified for 45°N latitude: CH₄, upward flux of 1.5×10^{11} molecules cm⁻² s⁻¹ (which results in a surface mixing ratio of 1.65 p.p.m.v., the present-day value); CO, upward flux of 6.0×10^{11} molecules cm⁻² s⁻¹ (see below); NO_x (NO + NO₂), upward flux 5.0×10^9 molecules cm⁻² s⁻¹; HNO₃, downward flux of 1.55×10^9 molecules cm⁻² s⁻¹; and O₃, fixed-number density of 7.5×10^{11} molecules cm⁻³. At the tropopause (10 km), the following non-zero boundary conditions were imposed: NO_x, downward flux across tropopause of 2.0×10^7 molecules cm⁻² s⁻¹; HNO₃, downward flux across tropopause of 1.2×10^8 molecules cm⁻² s⁻¹; and O₃, fixed number density of 1.0×10^{12} molecules cm⁻³. The boundary condi-

tions for NO_x , HNO_3 and O_3 are typical values used in most photochemical models¹⁰.

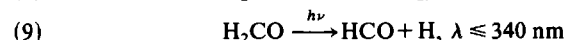
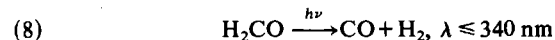
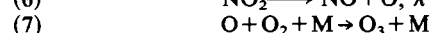
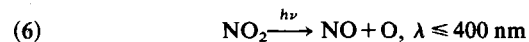
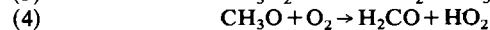
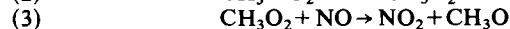
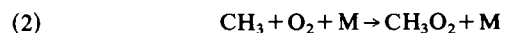
Methane is produced at the Earth's surface primarily by biological fermentation in anaerobic environments such as wetlands and rice fields; in ruminating animals, mostly cattle; and by biomass burning and natural gas leakage¹⁸. Methane is not produced photochemically or chemically in our strongly oxidizing atmosphere. Carbon monoxide is produced photochemically in the atmosphere via the methane oxidation chain¹⁹⁻²³. The oxidation of methane may be the largest source of CO, and may account for as much as 400–1,000 megatonnes of CO yr^{-1} (ref. 10). CO also results from other natural and anthropogenic activities. The estimated range for other known sources of CO (in units of $\text{Mt}(\text{CO}) \text{ yr}^{-1}$) are: oxidation of natural nonmethane hydrocarbons = 280–1,200; fossil fuel use = 400–1,000; forest clearing = 200–800; savanna burning = 100–400; emissions by plants = 50–200; oxidation of anthropogenic nonmethane hydrocarbons = 0–180; wood used as fuel = 25–150; ocean = 20–80; and forest wildfires = 10–50 (ref. 10). The range for the sum of the above CO sources other than the methane oxidation chain component, is 1,085–4,060 $\text{Mt}(\text{CO}) \text{ yr}^{-1}$ (equivalent to about $2.6 \times 10^{11} \text{ CO molecules cm}^{-2} \text{ s}^{-1}$). In a recent photochemical study, surface CO fluxes ranging between 1 and $10 \times 10^{11} \text{ CO molecules cm}^{-2} \text{ s}^{-1}$ were used²⁴. In our photochemical calculations for the present-day troposphere, we have assumed a surface CO flux which appears appropriate for 45°N, of $6 \times 10^{11} \text{ CO molecules cm}^{-2} \text{ s}^{-1}$. The Jungfraujoch spectra were obtained at a latitude of 46.5°N, just about the latitude of maximum CO concentration in the troposphere¹⁰. The CO produced via the methane oxidation chain is calculated explicitly as a photochemical production component in the model and is not included in the CO surface flux.

The apparent increase in tropospheric levels of CH_4 and CO over the past 35 years may have resulted from the following factors: (1) an increase in the global production rate of either, or both CH_4 and CO; (2) a decrease in the global destruction rate of either, or both CH_4 and CO; or (3) a combination of both (1) and (2), since both CH_4 and CO are closely coupled photochemically via the methane oxidation chain and through the hydroxyl radical (OH), the dominant destruction mechanism for both CH_4 and CO in the troposphere.

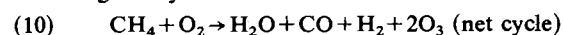
As mentioned above, the methane oxidation chain, which is initiated by the reaction of CH_4 with OH, and leads to the production of CO via the photolysis of formaldehyde (H_2CO), photochemically couples CH_4 and CO ¹⁸⁻²³.



The reaction of CH_4 with OH is the dominant destruction mechanism for CH_4 in the troposphere, and is also a significant loss mechanism for tropospheric OH. The methyl radical (CH_3) formed in reaction (1) continues the methane oxidation chain, which is illustrated in the following reactions:



The methane oxidation chain leads to the photochemical production of CO, H_2 and O_3 , and may be represented by the following net cycle:



Just as the reaction with OH (reaction 1) is the dominant destruction mechanism for CH_4 , the destruction of CO is also controlled by its reaction with OH:



The reactions of OH with CH_4 (reaction 1) and CO (reaction 11) control the global destruction of OH, the dominant oxidizing species in the troposphere, and, hence, the dominant tropospheric scavenging species. The reactions of OH with CH_4 and CO account for about 97% of the total tropospheric destruction of OH (for noontime, groundlevel conditions at 45°N, CO accounts for 81% and CH_4 16% of the total destruction of OH)¹⁰. The reaction between CH_4 and OH is such a dominant loss mechanism for CH_4 that more than 90% of the global destruction of CH_4 occurs in the troposphere, with nearly 80% occurring in the lowest atmospheric scale height¹⁸. Several model studies have considered the photochemical coupling of CH_4 , CO, and OH and the effects of anthropogenic emissions of CO on the CH_4 –CO–OH chemical system²⁴⁻²⁶.

Photochemical calculations can determine the surface flux of CH_4 (or CO) required to maintain a particular tropospheric mixing ratio of CH_4 (or CO). The critical parameter in these calculations is the vertical distribution of OH throughout the troposphere. Our photochemical calculations indicate that at 45°N (neglecting advection), the measured mixing ratio of CH_4 (1.65 p.p.m.v.) is maintained by a surface flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ ($645 \text{ Mt}(\text{CH}_4) \text{ yr}^{-1}$) and that a CO surface flux of $6 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ ($4,200 \text{ Mt}(\text{CO}) \text{ yr}^{-1}$) results in a surface mixing ratio of about 180 parts per 10^9 by volume (p.p.b.v.) decreasing to about 130 p.p.b.v. at 5 km. For this calculation, the OH concentration was found to decrease from $2.9 \times 10^6 \text{ cm}^{-3}$ at the surface to $2.0 \times 10^6 \text{ cm}^{-3}$ at 1 km and then range between 1.0 and $1.5 \times 10^6 \text{ cm}^{-3}$ between 2 and 10 km. The total OH column in the troposphere was $1.6 \times 10^{12} \text{ cm}^{-2}$. The calculation of the OH profile in the troposphere depends on several parameters, including the level of incident solar radiation, which photodissociates tropospheric ozone producing excited oxygen atoms, which then react with water vapor producing OH. The level of incident radiation available for the photodissociation of ozone is a function of season (solar declination), latitude, time of day, and the scattering properties of the atmosphere, including the surface albedo¹². Time-dependent calculations indicate that at the surface at 45°N, the noontime OH concentrations are about $2 \times 10^6 \text{ cm}^{-3}$ for a surface albedo of 10%¹⁰. Photochemical calculations indicate that the concentration of OH increases as the surface albedo is increased¹². For the calculations in this work we have assumed summer conditions (solar declination of 22.5°N) at 45°N with a surface albedo of 25%. These parameters tend to maximize the calculated values of OH. Our calculated OH values are consistent with time-dependent calculations¹⁰ and with calculations with a varying surface albedo¹².

We also investigated the sensitivity of the CH_4 –CO–OH system to changes in the prescribed surface fluxes of CO and CH_4 . We varied the surface CO flux from 4 to $8 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ (at 45°N, the CO mixing ratio is maintained by a surface CO flux of $6 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ and a surface CH_4 flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$). In all of these calculations, we used a surface CH_4 flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$. The surface concentrations of CO, CH_4 , and OH as a function of the surface CO flux are summarized in Table 1. These calculations

Table 1 Sensitivity of CO, CH_4 and OH to the CO surface flux

CO surface flux ($\text{molecules cm}^{-2} \text{ s}^{-1}$)	Surface mixing ratio		
	CO (p.p.b.v.)	CH_4 (p.p.b.v.)	Surface density OH (cm^{-3})
4×10^{11}	107.0	1.321	3.46×10^6
5×10^{11}	141.1	1.479	3.14×10^6
6×10^{11} *	180.9	1.654	2.85×10^6
7×10^{11}	226.3	1.840	2.60×10^6
8×10^{11}	276.6	2.027	2.38×10^6

For all of these calculations, a CH_4 surface flux of $1.5 \times 10^{11} \text{ molecules cm}^{-2} \text{ s}^{-1}$ was used.

* Standard present-day run.

Table 2 Sensitivity of CH₄, CO and OH to the CH₄ surface flux

CH ₄ surface flux (molecules cm ⁻² s ⁻¹)	Surface mixing ratio		
	CH ₄ (p.p.m.v.)	CO (p.p.b.v.)	Surface density OH (cm ⁻³)
1.0 × 10 ¹¹	0.984	155.0	3.14 × 10 ⁶
1.5 × 10 ¹¹ *	1.654	180.9	2.85 × 10 ⁶
2.0 × 10 ¹¹	2.398	205.6	2.64 × 10 ⁶

For all of these calculations, a CO surface flux of 6 × 10¹¹ molecules cm⁻² s⁻¹ was used.

* Standard present-day run.

tions indicate that if the CO surface flux is doubled (from 4 to 8 × 10¹¹ molecules cm⁻² s⁻¹), the surface CO mixing ratio increased by a factor of 2.6, the surface CH₄ mixing ratio increased by a factor of 1.5 and the OH number density decreased to 69% of its original value. For comparison, in an earlier study²⁴, it was found that by doubling the CO surface flux (from 5 to 10 × 10¹¹ molecules cm⁻² s⁻¹), the surface CO mixing ratio increased by a factor of 2.7, the surface CH₄ mixing ratio increased by a factor of 1.6, and the surface OH number density decreased to 62% of its original value. Similar results were also found in this study for a doubling of the CO surface flux from 1 to 2 × 10¹⁰ molecules cm⁻² s⁻¹ (ref. 24).

We also varied the surface CH₄ flux from 1.0 to 2.0 × 10¹¹ molecules cm⁻² s⁻¹ while holding the surface CO flux constant at 6 × 10¹¹ molecules cm⁻² s⁻¹. The surface concentrations of CH₄, CO, and OH as a function of the surface CH₄ flux are summarized in Table 2. These calculations indicate that a doubling of the surface CH₄ (from 1 to 2 × 10¹¹ molecules cm⁻² s⁻¹) results in an increase in the surface CH₄ mixing ratio of a factor of 2.4, an increase in the surface CO mixing ratio of a factor of 1.3, and a decrease in the surface OH number density to 84% of its original value.

Decreased levels of CH₄ and CO in the 1950–51 troposphere may be attributable to a decreased surface flux of CH₄ and/or CO, due to the chemical coupling via the methane oxidation chain and OH destruction. In our calculations, we have assumed that surface fluxes of both CH₄ and CO have increased as a result of anthropogenic perturbations to the production of both gases. Calculations were performed to determine the fluxes of both CH₄ and CO consistent with the lowered CH₄ and CO mixing ratios deduced from the 1950 and 1951 groundbased solar infrared spectra, that is, CH₄ = 1.14 p.p.m.v. and CO about 65 p.p.b.v. in the free troposphere (5 km), which is within the uncertainty in the spectral determination (76 ± 25 p.p.b.v.)². Our results indicate that a CH₄ mixing ratio of 1.14 p.p.m.v. is maintained by a flux of 1.35 × 10¹¹ cm⁻² s⁻¹ (580 Mt(CH₄) yr⁻¹) and the CO mixing ratio is maintained by a flux of 4.0 × 10¹¹ cm⁻² s⁻¹ (2,800 Mt(CO) yr⁻¹). These calculations indicate that while the CH₄ mixing ratio increased about 44% from 1951 to the present (1.14 p.p.m.v. to 1.65 p.p.m.v.), the 45° N CH₄ flux increased by only 11% (1.35 to 1.50 × 10¹¹ molecules cm⁻² s⁻¹). During this same period, the CO free tropospheric (5 km) mixing ratio increased by about 100% (from about 65 to about 130 p.p.b.v.), while the 45° N CO flux only increased by 50% (4.0 to 6.0 × 10¹¹ molecules cm⁻² s⁻¹). For this calculation, the OH concentration was found to decrease from 3.6 × 10⁶ cm⁻³ at the surface to 2.6 × 10⁶ cm⁻³ at 1 km and then range between 1.3 and 1.9 × 10⁶ cm⁻³ between 2 and 10 km. The total OH column in the troposphere was 2.1 × 10¹² cm⁻².

Photochemical calculations indicate at 45° N, the OH column in the troposphere decreased by about 25% (from 2.1 × 10¹² to 1.6 × 10¹² cm⁻²) from 1950 to the present, as a result of increased levels of CH₄ and CO in the troposphere. It is interesting to note that in a recent paper, a decrease in tropospheric OH of between 20 and 30% over the last 130 years was estimated as a result of increasing levels of CH₄²⁷. What is particularly interesting is that this estimate was based on two entirely different methods of analysis (on a mass balance of CH₄ in the

Table 3 Sensitivity of calculated CH₄, CO and OH to reduced NO_x and O₃ in 1950–51 troposphere

O ₃ Reduction	Reduced O ₃ (fixed NO _x)		Surface OH
	Surface CH ₄	CO at 5 km	
-10%	+6%	+8%	-8%
-25%	+17%	+20%	-19%
-50%	+40%	+47%	-38%

NO _x Reduction	Reduced NO _x (fixed O ₃)		Surface OH
	Surface CH ₄	CO at 5 km	
-10%	+2%	+2%	-1%
-25%	+5%	+5%	-6%
-50%	+13%	+13%	-17%

Values are calculated percentage change from standard 1950–51 calculation. In a standard run, the surface O₃ mixing ratio is 29.5 p.p.b.v. and the surface NO_x mixing ratio is 0.5 p.p.b.v.

past and present atmosphere and on calculations of the lifetimes of CH₄ in the past and present atmosphere), neither of which involve photochemical calculations, as does the present analysis. Hence, increasing levels of CH₄ and CO clearly impacted the scavenging ability of OH, the major chemical scavenger in the troposphere.

The calculated concentrations of CH₄, CO and OH are sensitive to the tropospheric levels of the nitrogen oxides, NO_x (NO + NO₂) and ozone (O₃)^{10,11}. Unfortunately, the 1950–51 spectra used to deduce levels of CH₄ and CO can provide no information on tropospheric NO_x and O₃. In fact, there does not appear to be any data base to provide this information. However, we can perform photochemical calculations to determine the sensitivity of the calculated concentrations of CH₄, CO and OH to changes in NO_x and O₃. It appears that both NO_x and O₃ in the troposphere are impacted by anthropogenic activities^{10,11}, and, hence we would expect lower concentrations in the 1950–51 time period. Therefore, we reduced present-day levels of NO_x by 10%, 25% and 50%, while keeping O₃ at the present-day level and then reduced present-day levels of O₃ by 10%, 25% and 50%, while keeping NO_x at the present-day level. The sensitivity of calculated levels of CH₄ and CO on other tropospheric species is summarized in reactions (1) to (11). The concentration of tropospheric OH is given by the following expression, with the OH production terms in the numerator, and the OH destruction terms in the denominator¹⁰:

$$(12) \quad [\text{OH}] = \frac{2k[\text{O}(^1\text{D})][\text{H}_2\text{O}] + [\text{HO}_2]\{k[\text{O}_3] + k[\text{NO}]\} + 2J[\text{H}_2\text{O}_2]}{k[\text{CO}] + k[\text{CH}_4]}$$

In this equation, k is the kinetic chemical reaction rate and J is the photolysis rate of H₂O₂. In the lower tropical troposphere, the production of OH occurs mainly by the first term in the numerator, with smaller contributions from the other three terms. Contributions from O₃ and NO grow in importance with latitude reflecting an increase in O₃ and NO due to anthropogenic activities. The photolysis of O₃ leads to the production of excited oxygen atoms (O(¹D)), which react with water vapour forming OH. Hence, OH production increases with increasing levels of O(¹D) and H₂O, as indicated by the first OH production term in the numerator. The concentration of OH is independent of NO for NO levels below about 10 parts per 10¹² by volume (p.p.t.v.); OH increases as a function of NO between 10 and 500 p.p.t.v.; and decreases as NO increases above about 1 p.p.b.v.¹⁰ The production of OH via the photolysis of hydrogen peroxide (H₂O₂), represented by the fourth term in the numerator, is a significant source of OH in the upper troposphere¹⁰. Also note that the loss of OH is controlled by its reactions with CH₄ and CO, as shown in the denominator. The results of these sensitivity calculations for reduced tropospheric levels of O₃ and NO_x are summarized in Table 3. Inspection of this table indicates that surface OH concentrations decrease with reduced levels of O₃ and NO_x, as predicted by equation

(12). For all of the reduced levels of O_3 and NO_x , except for the 50% O_3 reduction case, the decrease in OH is less than 20% of the standard 1950 calculation, indicating that OH levels may have indeed decreased from 1950 to the present day, as CH_4 and CO levels increased. The variation of OH as a function of NO_x level summarized in Table 3 is in good agreement with earlier sensitivity calculations^{10,24}.

Photochemical calculations of present-day levels of CH_4 and CO compared to levels of these gases deduced from 1950 and 1951 ground-based solar infrared spectra^{1,2} suggest that at 45° N: (1) the lower surface flux of CH_4 may have increased by about 11% from 1951 to the present, although the CH_4 mixing ratio increased by about 44%; (2) the lower surface flux of CO may

have increased by about 50% from 1950–51 to the present, although the free tropospheric (~5 km) CO mixing ratio increased by about 100%; and (3) increased tropospheric levels of CH_4 and CO from 1950–51 to the present resulted in about a 25% decrease in the tropospheric column of OH, and, hence, impacted the ability of the major tropospheric chemical scavenger to scavenge. Finally, the analysis presented here suggests that historic spectra coupled with photochemical calculations may provide important and previously unattainable information on global production of atmospheric gases over time periods of decades. This information may permit an assessment of the role of anthropogenic emissions to the global budgets of atmospheric species over these time periods.

Received 20 May; accepted 25 September 1985.

1. Rinsland, C. P., Levine, J. S. & Miles, T. *Nature* **318**, 245–249 (1985).
2. Rinsland, C. P. & Levine, J. S. *Nature* **318**, 250–254 (1985).
3. Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **86**, 9826–9832 (1981).
4. Seiler, W. *2nd Symposium on the Composition of the Non-Urban Troposphere* (American Meteorological Society, Boston, 1982).
5. Blake, D. R. *et al. Geophys. Res. Lett.* **9**, 477–480 (1982).
6. Khalil, M. A. K. & Rasmussen, R. A. *J. geophys. Res.* **88**, 5131–5144 (1983).
7. Craig, H. & Chou, C. C. *Geophys. Res. Lett.* **9**, 1221–1224 (1982).
8. Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **89**, 11599–11605 (1984).
9. Khalil, M. A. K. & Rasmussen, R. A. *Science* **224**, 54–56 (1984).
10. Logan, J. A., Prather, M. J., Wofsy, S. C. & McElroy, M. S. *J. geophys. Res.* **86**, 7210–7254 (1981).
11. Levine, J. S. & Allario, F. *Envir. Monitor Assess.* **1**, 263–306 (1982).
12. Augustsson, T. R. & Levine, J. S. *Atmos. Envir.* **16**, 1373–1380 (1982).
13. Brewer, D. A., Augustsson, T. R. & Levine, J. S. *J. geophys. Res.* **88**, 6683–6695 (1983).
14. Brewer, D. A., Ogliaruso, M. A., Augustsson, T. R. & Levine, J. S. *Atmos. Envir.* **18**, 2723–2744 (1984).
15. Levine, J. S., Augustsson, T. R., Anderson, I. C., Hoell, J. M. & Brewer, D. A. *Atmos. Envir.* **18**, 1797–1804 (1984).
16. Anderson, D. E. & Meier, R. E. *Appl. Opt.* **18**, 1955–1960 (1979).
17. Elterman, L. *Envir. Res. Pap.* **285**, 1–48 (U.S. Air Force Cambridge Research Laboratory, Bedford, Massachusetts, 1968).
18. Wofsy, S. C. *A. Rev. Earth planet. Sci.* **4**, 441–469 (1976).
19. Levy, H. *Science* **173**, 141–143 (1971).
20. Levy, H. *Planet. Space Sci.* **20**, 919–931 (1972).
21. Levy, H. *J. geophys. Res.* **78**, 5325–5332 (1973).
22. McConnell, J. C., McElroy, M. B. & Wofsy, S. C. *Nature* **233**, 187–188 (1971).
23. Wofsy, S. C., McConnell, J. C. & McElroy, M. B. *J. geophys. Res.* **77**, 4477–4493 (1972).
24. Callis, L. B., Natarajan, M. & Boughner, R. E. *J. geophys. Res.* **88**, 1401–1426 (1983).
25. Chameides, W. L., Liu, S. C. & Ciccone, R. J. *J. geophys. Res.* **82**, 1795–1798 (1977).
26. Sze, N. D. *Science* **195**, 673–675 (1977).
27. Khalil, M. A. K. & Rasmussen, R. A. *Atmos. Envir.* **19**, 397–407 (1985).

Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*

Ronald H. A. Plasterk*, Melvin I. Simon* & Alan G. Barbour†

* California Institute of Technology, Division of Biology 147-75, Pasadena, California 91125, USA

In Borrelia hermsii, a spirochaete that causes relapsing fever, the switch between expression of two frequent variable major protein (VMP) types (7 and 21) is associated with a DNA rearrangement. Both cell types 7 and 21 contain untranscribed 7 and 21 VMP genes on linear plasmids. The serotype 7 cells contain an additional copy of the 7 VMP gene fused to an expression sequence on another linear plasmid. Switching to the 21 serotype involves removal of the transcribed 7 VMP gene and fusion of a copy of the 21 VMP gene to this same expression sequence. Thus recombination between linear plasmids can activate different VMP genes.

PATHOGENIC microorganisms have strategies to escape the host's antibody response. The selective pressure to develop evasive mechanisms is especially strong for extracellular pathogens that cycle between the vertebrate bloodstream and haematophagous arthropods: the longer the parasite survives in the bloodstream, the greater the probability of transmission through an arthropod to another animal. One of the strategies that these pathogens has developed is antigenic variation: a small fraction of multiplying cells undergoes a genetic change leading to the synthesis of a new and antigenically distinct surface protein. Those cells possessing novel serotypic determinants escape the immune response that is directed against the infecting serotype. The consequence to the host of antigenic variation is a relapsing or chronic illness.

Multiphasic antigenic variation has been described in eukaryotic and prokaryotic organisms. Salivarian trypanosomes such

as *Trypanosoma brucei* have a large assortment of surface glycoprotein genes that can be expressed in the host¹. Surface proteins of the bacterium *Neisseria gonorrhoeae* can vary during infection of human mucosa². Another group of bacteria, the borreliae, change their surface antigens during the course of relapsing fever and, like trypanosomes, are blood-borne and transmitted by vector³.

We are studying the molecular basis for antigenic variation in a *Borrelia* sp. for several reasons. (1) The antigenic repertoire is elaborate: 26 variant serotypes have derived from one serotype^{4,5}. (2) Infection in mice can be started with a single *Borrelia*⁴. (3) The organism can be cultivated *in vitro*⁶. (4) The variable antigens are abundant surface proteins that differ extensively in their primary structures^{7–9}. (5) Antigenic variation has been observed *in vivo* and *in vitro* at a frequency of 10^{-4} (ref. 4). These properties allow the isolation of populations of clonal origin, development of specific antibody and nucleic acid probes, and analysis of genetic material before and after the antigenic switch.

Recently, it was demonstrated that switching between three

† Laboratory of Pathobiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana 59840, USA.

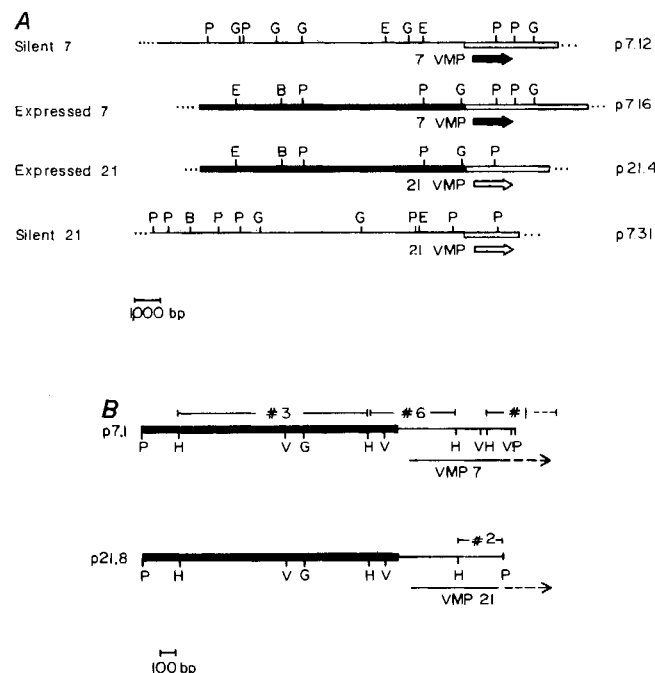


Fig. 1 **A**, The silent and expressed 7 and 21 VMP genes and surrounding sequences. The figure shows restriction maps of the clones p7.12 (silent 7 VMP from the 7 gene bank), p7.7 and p7.16 (overlapping clones containing expressed 7 VMP), p21.4 (expressed 21 VMP) and p7.31 (silent 21 VMP from the 7 gene bank). The sites indicated are: P, *Pst*I; G, *Bgl*II; B, *Bam*HI; E, *Eco*RI. The 1.9-kbp *Pst*I fragment downstream of the expressed 21 VMP gene (in p21.4) was used as a probe in Southern analysis of p7.16, and no homology was detected (not shown). Thus, in the DNA segments shown here the sequences downstream of the expressed 7 and 21 VMP genes are not identical (see text for discussion of the possible recombination reactions). The phages were selected as follows: the phages containing the 7 VMP gene (such as 7.7 and 7.16) were selected using as a probe an oligonucleotide specific for the 7 VMP structural gene (the α probe in ref. 10). The phages containing the expressed 21 VMP gene were selected using as a probe restriction fragment 3 (see **B**); this fragment is part of the expression sequence, and was subcloned from p7.16. The silent 21 VMP clones (such as 7.31 shown here) were selected using probe 2 (see **B**), a restriction fragment internal to the 21 VMP gene which was subcloned from p21.4. The restriction pattern of a clone containing the silent 7 gene selected from a library of serotype C DNA (pC.9) is identical to that of p7.12. This clone was included in the experiment described in Fig. 2A. **B**, The expressed VMP genes and the expression sequence. The figure shows restriction maps of the 2.9- and 2.8-kbp *Pst*I fragments that are subclones of p7.16 and p21.4, respectively. These fragments contain the 5' segment of the 7 and 21 VMP genes and part of the sequence upstream of these genes. These fragments have been cloned into the *Pst*I site of pBR322 (resulting in the clones p7.1 and p21.8). H, *Hind*III; V, *Pvu*II. The heavy line indicates the DNA that is homologous in both restriction fragments; this was determined by comparison of the restriction maps and by Southern blot analysis. The thin lines indicate the DNA that is specific for either the 7 or the 21 serotype. The VMP 7 and VMP 21 open reading frames are indicated by a horizontal arrow. For the 21 VMP gene the approximate start of the gene could be derived from the size of the truncated gene product produced by the recombinant p21.8 (see Fig. 2A). The restriction fragments that were used as probes in subsequent hybridization experiments were designated 1 (in the VMP 7 structural gene), 3 (in the expression sequence) and 2 (in the VMP 21 structural gene). These restriction fragments were cloned into the *Hind*III site of M13mp11, and total DNA of the recombinant clone was used as a probe in the subsequent hybridizations (M13 itself does not show any significant homology with *Borrelia* sequences; not shown). Fragment 1 was subcloned using *Hind*III. Therefore, the subcloned fragment contains not only *Borrelia* DNA (the DNA between the *Hind*III site and the *Pst*I site) but also 780 bp of pBR322 DNA (between the *Pst*I and the *Hind*III site). The relative orientation of this fragment cloned in M13mp11 could be determined by a *Pst*I digestion (as there is a *Pst*I site in the mp11 polylinker). We isolated single-stranded DNA of this recombinant and determined that an oligonucleotide containing VMP 7 structural gene antisense sequences (the α -oligonucleotide¹⁰) would hybridize to the plus strand of the recombinant. This confirms that the 7 VMP gene reads as indicated in the map. The clone p7.1 is similar to the previously described clone pRML7.1 (ref. 10); the only difference is the orientation of the insert in the *Pst*I site of pBR322. It was reported that pRML7.1 does not express a truncated VMP⁷¹⁰. However, we found that p7.1 does express a truncated protein; apparently, the expression of the truncated protein in pRML7.1 is hindered by the transcription of the β -lactamase gene on the other strand.

variable major proteins (VMPs) in *Borrelia hermsii* was associated with DNA rearrangements and with occurrence of expression-linked copies of all, or part, of a VMP-encoding gene¹⁰. In the present study we define the nature of the DNA rearrangement. We examined *B. hermsii* HS1 serotype 7, a serotype highly prevalent during early infections of mice^{4,5}, and serotype 21, which was derived from a serotype 7 cell line⁵. Use of these serotypes allowed analysis of DNA structure before and after the switch to serotype 21 from 7. We constructed gene libraries of serotype 7 and 21 DNA, selected clones containing the sequences that are involved in the rearrangement, and analysed the nature of the rearrangement associated with the switch from 7 to 21. A third serotype, C, was included in some experiments. Serotype C has been observed in cultures in defined medium but never spontaneously in mice^{4,7}. Antigenic switching of serotype C cells to another serotype has not been observed in defined medium or after infection of mice (A.G.B. unpublished observations).

Silent and expressed VMP genes

DNA from serotypes 7 and 21 was partially digested with *Sau*3A, and fragments of ~15 kilobase pairs (kbp) were cloned into phage λ EMBL3 (ref. 11). Phages that contained the sequences coding for the 7 and 21 VMP genes were selected using probes previously shown to be either specific for the 7 VMP gene or associated with the 21 VMP gene (ref. 10; see legend to Fig. 1A). *Borrelia* DNA was subsequently subcloned into the *Sall* site of pBR322. We used monoclonal antibodies specific for VMPs 7 and 21 (ref. 9) to determine whether the proteins were expressed by the recombinant clones in *Escherichia coli*. As shown in Fig. 2A, some of the clones containing the 7 VMP gene expressed the protein; others did not. This difference between clones in expression of the VMP is not a cloning artefact. Analysis of the restriction maps of the expressing and the non-expressing DNA (see Fig. 1A) shows that the sequences upstream of the 7 VMP gene in the clones that do express VMP 7 are completely different from those in the clones that do not express it. Similarly, the serotype 21 clones can be divided into two classes: those that express VMP 21 and those that do not. Each class has a characteristic restriction pattern. The clones contain identical 21 VMP gene regions, but the sequences upstream of the genes differ between clones that express VMP 21 and clones that are 'silent'.

Identical 5'-end sequences in expressed genes

The restriction map upstream of the expressed and silent VMP genes is different. However, comparison of the restriction maps of the VMP 7-expressing clones and the VMP 21-expressing clones shows that the restriction sites upstream of the two genes are identical. Thus, the expression of these genes may result from the juxtaposition of the common DNA sequence and the structural VMP gene, and it seems appropriate to call this common sequence the 'expression sequence'.

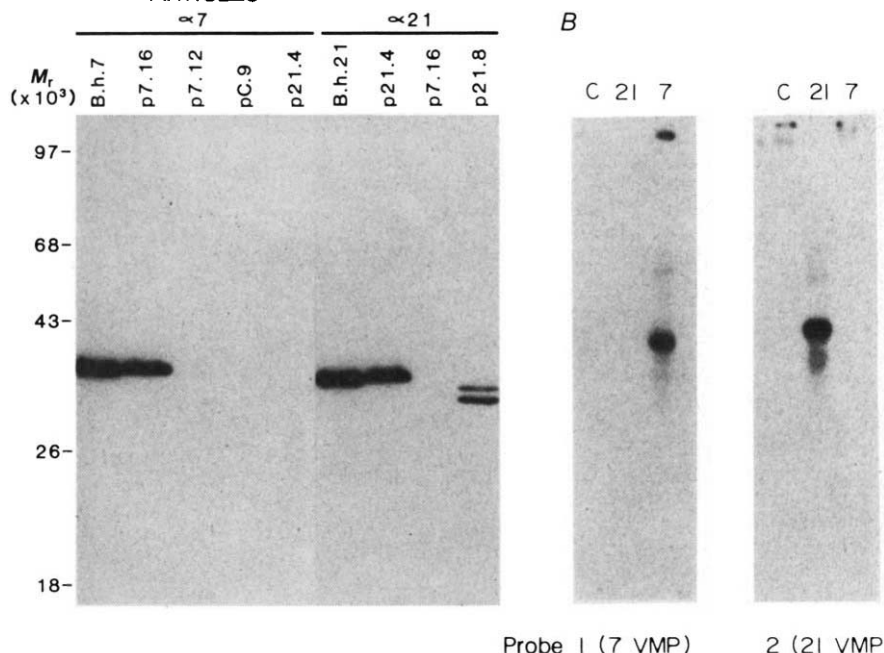
To demonstrate further the correlation between gene expression and juxtaposition to the expression sequence, we subcloned the regions containing the site in which the expression sequence is fused to either the 7 or the 21 VMP DNA. Figure 1B shows a restriction map of a 2.9-kbp *Pst*I fragment containing the expression sequence DNA and part of the 7 VMP gene (p7.1), and a 2.8-kbp *Pst*I fragment containing the identical regions of the expression sequence and part of the 21 VMP gene (p21.8). The sequences indicated by a solid bar are identical in both fragments; those indicated by a thin line are different.

To prove that the regions adjacent to the expression site corresponded to part of the VMP 7 and 21 genes, we used restriction fragments 1 (from the 7 clone) and 2 (from the 21 clone) as probes in Northern analysis of RNA from *Borrelia* serotypes 7 and 21. Figure 2B shows that probe 1 specifically hybridizes to the messenger RNA for VMP 7, and probe 2 hybridizes to the mRNA for VMP 21, demonstrating that these DNA fragments are internal to the VMP 7 and 21 genes.

Similar conclusions were drawn from Western blot analysis of *E. coli* strains containing p7.1 or p21.8. Figure 2A shows that

Fig. 2 A. Western blot analysis of lysates of *B. hermsii* or of *E. coli* containing recombinant plasmids, with *Borrelia* DNA. Monoclonal antibody H12436 (9) is anti-VMP 7 ($\alpha 7$; left-hand panel); monoclonal antibody H10022 (9) is anti-VMP 21 ($\alpha 21$; right-hand panel). Lanes contained lysates of *B. hermsii* serotypes 7 (B.h.7) or 21 (B.h.21) or lysates of *E. coli* JM101 containing p7.16, p7.12, pC.9, p21.4 or p21.8. On the left are shown the relative migrations of pre-stained M_r standards (6041SA; BRL). *B. hermsii* cells were cultured, collected and lysed as described previously^{9,10}. The *E. coli* were grown in CYHM medium (1% Difco casamino acid, 0.5% Difco Bacto yeast extract, 50 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$ and 20 mM HEPES pH 7.4). Harvested cells were resuspended in phosphate-buffered saline containing 5 mM $MgCl_2$ and 20 $\mu g\ ml^{-1}$ of phenylmethylsulphonyl fluoride and incubated for 15 min at 25 °C, then centrifuged. The pellet was resuspended in sample buffer containing 1% SDS and boiled for 5 min. The lysate was cleared by centrifugation; lysate components were analysed by Western blot analysis as described previously⁹. The blocking and antibody incubation solution comprised 2% bovine serum albumin in 50 mM Tris-HCl pH 7.6, 150 mM NaCl, 5 mM EDTA (TSE); the washing and ^{125}I -protein A incubation solution comprised 0.05% Tween 20 in TSE. **B.** Northern blot analysis of RNA from the three serotypes 7, 21 and C. The left-hand panel shows analysis of the three types of RNA using probe 1 (from the 7 VMP gene); this probe hybridizes to one major band that is present only in the 7 strain. Similarly, the probe from the 21 gene (2) hybridizes to a band of approximately the same size (1,100 bases) that is present only in the 21 strain. This result shows that the difference between the two strains in VMP expression is indeed at the transcriptional level, and that the probes used are indeed specific for the different VMP genes.

Methods. The experiments were done as follows: total *Borrelia* RNA was isolated and electrophoresed as described¹⁰, transferred to a Biodyne Nylon membrane and baked under vacuum. After 1 h of prehybridization, the blot was incubated at 37 °C for 16 h in 6 $\times$ SSC, 50% formamide, 1% SDS, 0.1 $\times$ Denhardt's and 100 $\mu g\ ml^{-1}$ sonicated *E. coli* DNA and the probe. Probes were made by nick-translation of the subclone DNA, using 40 μCi of [α - ^{32}P]dATP per reaction. Free nucleotides were removed by spinning the sample through a 200- μl BioRad P200 column. After hybridization the blots were rinsed at room temperature and washed in 2 $\times$ SSC, 1% SDS at 50 °C for 30 min. Exposure was for between 2 and 16 h at -70 °C with an intensifying screen.



recombinant p21.8 expresses a truncated VMP 21. Similarly, the recombinant p7.1 expresses a truncated VMP 7 (not shown). Thus, the *Pst*I site interrupts the coding sequence of both VMP genes. This result also indicates the direction of reading of the VMP genes, as the 5' end of the gene is apparently contained within the subcloned fragment.

Activation of VMP genes

The RNA analysis showed that the switch from serotype 7 to serotype 21 involves switching-off VMP 7 transcription and switching-on VMP 21 transcription. We showed that expression of the VMP genes in *E. coli* is associated with juxtaposition to a specific DNA segment. If the exclusive expression of one VMP gene is the result of the presence of that VMP gene adjacent to the expression sequence, then the switch from serotype 7 to 21 should involve the replacement of the 7 VMP gene adjacent to the expression sequence by the 21 VMP gene. The probes specific for the 7 or 21 VMP genes (1 and 2) were used to follow the fate of the 7 and 21 VMP genes in the switch.

Figure 3A, panel b shows *Pst*I-digested DNA from 7, 21 and C cells, hybridized with probe 1. All three strains contain a fragment of 8.5 kbp that hybridizes with the probe. The same fragment is found in clone p7.12, which does not express VMP 7; this suggests that the 8.5-kbp fragment represents the silent form of the 7 VMP gene in all serotypes. The 7 DNA contains an additional fragment of 2.9 kbp that hybridizes with the probe; this is the fragment found in clone p7.16 (the VMP 7-expressing clone). The expressed copy of the 7 VMP gene is no longer present after the switch to the 21 serotype. When the VMP 21-specific probe (2) was used, a 1,250-base pair (bp) *Pst*I fragment was observed in the three serotypes (Fig. 3A, panel c); this is the fragment present in clone p7.31 which contains the silent 21 VMP gene. In addition to this fragment, the 21 DNA contains a 2.8-kbp fragment that hybridizes with the probe, and this is

the fragment that is found in the VMP 21-expressing clone in which the 21 VMP gene is fused to the expression site. This result shows that the activation of the 21 VMP gene is also associated with a duplication of the gene and fusion to the expression sequence.

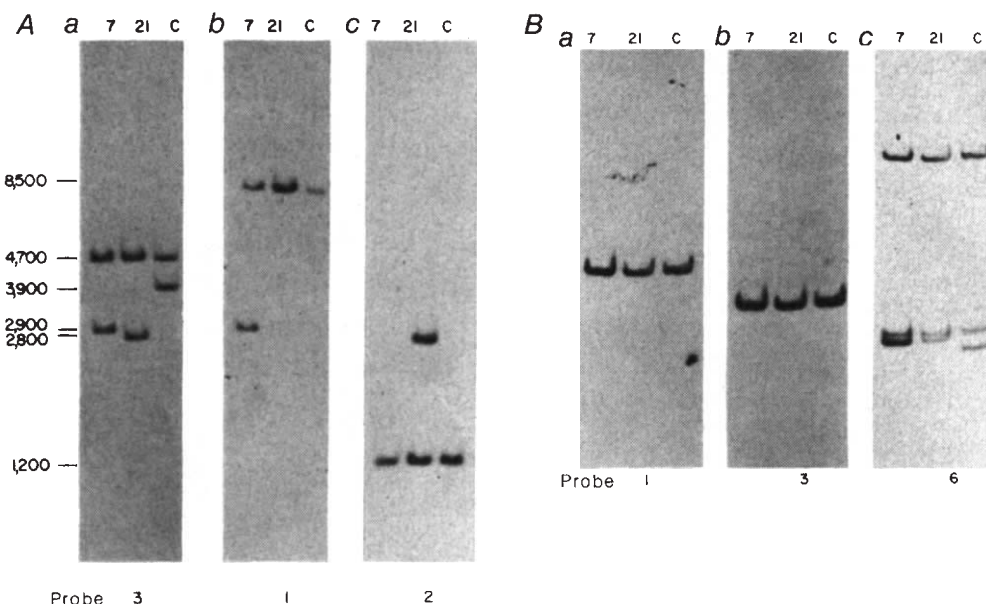
The above results suggest that in each serotype the expression sequence is fused to a different VMP gene. To demonstrate this, we used the expression sequence (fragment 3, Fig. 1B) as a probe in a Southern blot analysis; the fragments specific for the expressed 7 VMP gene and the expressed 21 VMP gene showed hybridization (2.9 and 2.8 kbp respectively; see Fig. 3A, panel a). In the C serotype the expression sequence is fused to yet another DNA segment (resulting in a 3.9-kbp *Pst*I segment). In addition, probe 3 hybridized to a 4.7-kbp fragment common to all serotypes (see legend to Fig. 3A).

Several conclusions can be drawn from these data: in the 7 serotypes two copies of the entire 7 VMP gene are found. One of these copies is the transcribed copy, and this is fused to the expression sequence. In addition, the cells contain an untranscribed copy of the 21 VMP gene. When the cells switch to serotype 21, the copy of the 7 VMP gene that is adjacent to the expression site is replaced by a copy of the 21 VMP gene.

The site at which the expression sequence and the VMP gene are fused can be referred to as the recombination site. In the expressed VMP clone this site was mapped as follows: the 2.9-kbp *Pst*I fragment containing the site was divided into *Hind*III fragments. Each of these fragments was used as a probe of *Hind*III-digested DNA from different serotypes. The *Hind*III fragment containing the recombination site should show a pattern that is different in the three strains. As shown in Fig. 3B, the fragment numbered 6 contains the recombination site. No other rearrangement was seen in this 2.9-kbp *Pst*I fragment.

We have shown that the activation of a VMP gene is the result of fusion of that gene to the expression site. We do not know

Fig. 3 A, Southern analysis of *Pst*I-digested DNA from the three serotypes 7, 21 and C. The probes were 3(a), 1(b) and 2(c). Probe 1 hybridizes to an additional band of 2.9 kbp in the 7 DNA; probe 2 hybridizes to an additional 2.8-kbp fragment in the 21 DNA. Note that probe 3 hybridizes to a 2.9-kbp fragment in the 7 DNA and also a 2.8-kbp band in the 21 DNA, as well as to a 3.9-kbp fragment in the C DNA. All three of these fragments are specific for each serotype. In addition, probe 3 hybridizes to a fragment of 4.7 kbp that is present in all three serotypes; this *Pst*I fragment has been mapped. The plasmids p7.7 and p21.4 (that is, the plasmids containing the expression site) contain a 4.7-kbp *Pst*I fragment that hybridizes with probe 3 (not shown). This *Pst*I fragment was mapped adjacent to the recombination site (see Fig. 1a). Nick-translation and hybridization were done as described in Fig. 2 legend, except that hybridization was done at 42 °C, and the final washing was at 65 °C in 0.1 × SSC, 1% SDS. The film was exposed for 2 h at -70 °C. **B**, Southern blot analysis of *Borrelia* DNA of three serotypes (7, 21 and C) digested with *Hind*III. **a**, The hybridization pattern obtained when subclone 1 was used as a probe (see Fig. 1). **b**, Same blot as **a** after removal of the first probe and hybridization with probe 3. **c**, Same blot again, but after hybridization with probe 6. The pattern using probes 1 and 3 is identical in all three lanes; that using probe 6 is different in each lane.



how the expression site activates the adjacent VMP gene; one possible explanation is that it contains a promoter for transcription reading into the adjacent DNA.

VMP genes carried on linear plasmids

We obtained an unusually high frequency of positive clones (5–10%) in the 7 and 21 phage banks, using probes that on Southern analysis of digested total DNA hybridized to only one or a few restriction fragments. If these genes were represented only once per genome this would predict an unusually small genome size. The most likely explanations are either that the genes are present in multiple copies in the chromosome of *B. hermsii* or that they are present on plasmids with a high copy number per cell. To resolve this issue we ran the total DNA on 0.5% agarose gels and observed several discrete bands. A better and quicker separation was achieved by pulse-field electrophoresis¹². The resolved bands were relatively small (~30 kbp) and optimal separation was obtained using short pulse times (1 s). Figure 4A shows total DNA from three *Borrelia* serotypes separated by pulse-field gel electrophoresis. The top band is the *Borrelia* chromosome (as a reference we included DNA from *E. coli* that was isolated using the same lysis and purification procedure; it co-migrated with the *Borrelia* chromosome). The banding patterns for serotypes 7 and 21 are very similar. Note, however, that the structure of the DNA of the C serotype is different—an extra band is visible. The fact that these molecules can be separated by pulse-field electrophoresis suggests that they are linear DNA molecules, as this technique does not resolve circular DNA well¹² (see below). The presence of extrachromosomal DNA does not seem to be unique to *B. hermsii*: as shown in Fig. 4A, the species *Borrelia burgdorferi*, the cause of Lyme disease, contains numerous bands. *E. coli* K-12 (Fig. 4A) and *Spirochaeta aurantia*, a free-living non-pathogenic spirochaete (not shown), yielded no detectable extrachromosomal DNA in this experiment. The simplest explanation for these bands is that they represent discrete extrachromosomal elements, that is, plasmids.

To determine whether these DNA elements contain the silent and expressed VMP genes, we probed the DNA separated by electrophoresis on a 0.7% agarose gel with probes for the 7 and

21 VMP genes and the expression site. As shown in Fig. 4B, the expression site lies on an extrachromosomal element of ~28 kbp in both the 7 and 21 strains. The VMP 7-specific probe hybridized to the 32-kbp plasmid in the 21 strain. Thus, the silent 7 VMP gene lies on a plasmid other than the one carrying the expression site. In the 7 strain two plasmids hybridized with the VMP 7 probe: one (32 kbp) containing the silent 7 VMP gene and the other (28 kbp) containing the expressed 7 VMP gene. In the 7 DNA the 28-kbp band hybridized with the VMP 2-specific probe. This result means that the silent 21 VMP gene is carried on a plasmid of the same size as the plasmid that carries the expression site. A clone of approximately 15 kbp containing the silent 21 gene (p7.31) contains no homology with clones of the expressed 7 gene (p7.7 and p7.16), which indicates that the silent 21 gene lies on a DNA molecule of 28 kbp that is different from the expression plasmid. This pattern shows that in the switch from type 7 to 21, the 7 VMP gene is removed from the 28-kbp plasmid, and a copy of the 21 VMP gene is inserted into the same site on the 28-kbp plasmid.

The behaviour of the extrachromosomal elements in pulse-field electrophoresis suggested that they might be linear rather than circular DNA molecules. We wanted to test whether the DNA of 28 kbp was indeed linear, and if so, whether the sequences on the molecule were circularly permuted. Therefore, we treated the putative ends of the DNA molecule with an exonuclease. If the DNA is linear and has specific ends, treatment with an exonuclease followed by restriction enzyme digestion and gel electrophoresis should result in a shift in the relative molecular mass (M_r) of specific restriction fragments. As we could obtain only small amounts of the 28-kbp DNA, we did the experiment as follows: total DNA from the 7 serotype was digested for different lengths of time with *Bal*31 nuclease. The DNA was then digested with *Pst*I and separated on a gel. The fragments contained in the 28-kbp band were visualized by Southern blotting and hybridization with nick-translated 28-kbp plasmid DNA. The result is shown in Fig. 5A: the probe hybridized to a series of *Pst*I fragments in the 7 DNA, but only one of these bands showed a shift down of M_r after treatment with *Bal*31 nuclease. Once this band had been degraded, a second band began to shift down. We concluded from this experiment

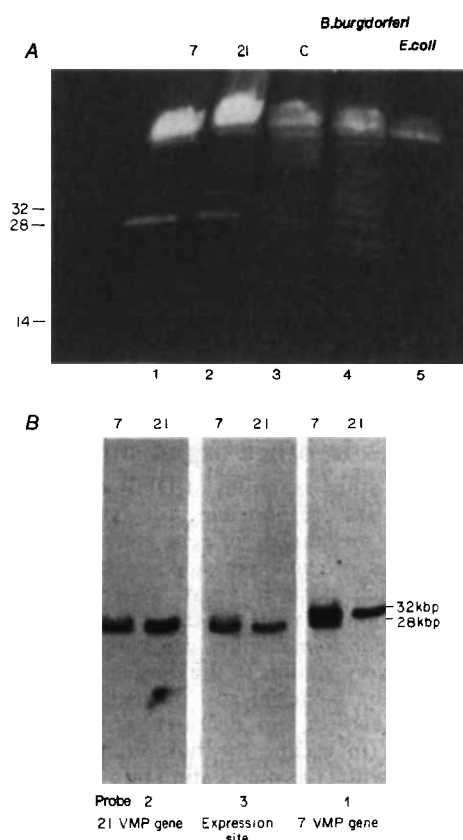


Fig. 4 **A**, Pulse-field gel electrophoresis of *Borrelia* DNA. DNA from *B. hermsii* and *E. coli* was isolated as described¹⁰ and separated as follows: a 1.1% agarose gel was placed in a tray with two pairs of electrodes (a slight modification of the original method described by Schwartz and Cantor¹², Stanford workshop); 10 μ l of DNA was loaded per lane and electrophoresis was at 175 V for 16 h, with the field changing every second. The gel was stained with ethidium bromide and photographed as any DNA/agarose gel. The top band is the chromosomal DNA (in lane 5, *E. coli* chromosomal DNA is shown). In addition, there are several extrachromosomal DNA elements visible. The pulse time (1 s) was chosen to optimize separation of the 28-kbp band from the 32-kbp band. When longer times are chosen the larger elements are separated better, and it is then clear that the band just beneath the chromosomal band is indeed separate. **B**, Southern analysis of the extrachromosomal *Borrelia* DNA. Total *Borrelia* DNA from the 7 and 21 serotypes was separated on a 0.7% agarose gel (25 V, 60 h at 4°C). The resulting pattern is identical to that shown in **A**. We chose standard separation in a low-percentage gel rather than in a high-percentage gel in pulse-field electrophoresis because the capillary transfer of large molecules is better from a low-percentage gel. However, the experiment has also been done after transfer from a pulse-field gel, or by hybridizing to the dried gel itself, with similar results. The gel was treated briefly with 0.25 M HCl, then the DNA was transferred as described above and hybridized with the probes as indicated. The same blot was used with all three probes.

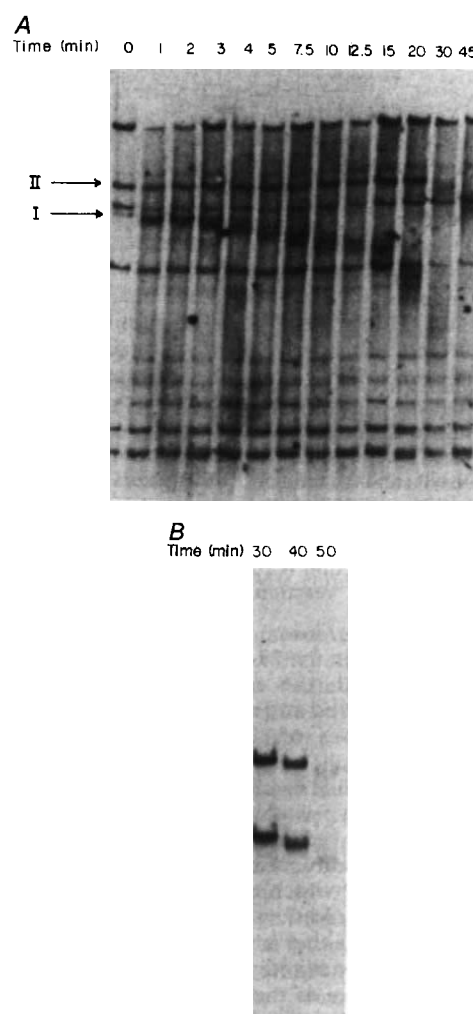


Fig. 5 *Bal31* degradation of the terminal region of the 28-kbp plasmid. **A**, *Borrelia* DNA (serotype 7) was treated for the indicated time with 0.15 units of *Bal31* (Boehringer Mannheim). EDTA was added to a final concentration of 20 mM, then the DNA was recovered by alcohol precipitation and digested with *Pst*I before separation on a 1% agarose gel. After blotting the gel was hybridized with '28-kbp probe'. This probe was obtained as follows: serotype 7 DNA was run out on a pulse-field gel, and after ethidium bromide staining the 28-kbp plasmid band was cut out of the gel, and the DNA recovered from it. Then it was nick-translated as described above. The arrow labelled I indicates the first band that disappears; the arrow labelled II indicates the second band. **B**, 21 DNA was treated in a time series with *Bal31* as described for **A**. As a probe we used clone 2 (see Fig. 1B), which is a restriction fragment internal to the 21 VMP gene. The two bands that are seen are the 1,250- and 2,800-bp fragments observed in Fig. 3A, corresponding to the silent and the expressed 21 gene respectively. The fragments were initially not affected during the time series. The timepoints are shown at which suddenly both bands began to shift down simultaneously.

that the 28-kbp band results from a linear DNA molecule having specific ends. We do not yet know in what form this molecule exists in the cell. It seems probable, however, that the ends of the DNA molecule are held together by other macromolecules in the cell (for example, proteins). As only one restriction fragment shifted in the gel in Fig. 5A, it seems that one of the ends of the plasmid was protected from degradation, for example by an amino acid or peptide that was covalently bound and was not removed by the protease and phenol treatment during the DNA purification. We have used nick-translated restriction fragments instead of the total 28-kbp DNA as probes for similar gels. Using the expression-linked 21 VMP gene as a probe (probe 2) with *Bal31* digestion of serotype 21 DNA, it was shown that this fragment is degraded late in the time series (see Fig. 5B),

hence the transcribed 21 VMP gene is located several kilobases away from the nuclease-sensitive end on a linear plasmid. Note that the bands corresponding to the silent and expressed 21 VMP genes shifted down at the same time; they are therefore at approximately the same distance from the *Bal31*-sensitive end of the plasmids, so the distance from the 21 VMP gene to the end of the plasmid downstream of the gene is not significantly changed by the recombination of the silent gene into the expression site.

Switch mechanism

A schematic representation of the molecular basis of antigenic variation is given in Fig. 6. We have shown for two VMP genes that expression results from the presence of a copy of the gene

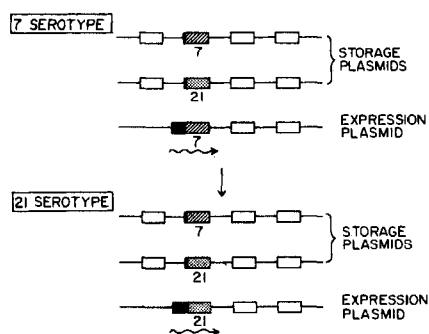


Fig. 6 Model for the molecular basis of antigenic variation in *Borrelia*. The relevant plasmids of the 7 and 21 serotypes are shown. The storage plasmids are identical in both strains. The 21 VMP gene in the 7 cells is not transcribed. It transposes into the expression site, and the resident 7 VMP gene is removed. The VMP gene that is adjacent to the expression sequence is transcribed. As discussed in the text, several mechanisms for the recombination reaction are possible, but we favour the one in which a reciprocal site-specific recombination is followed by loss of one of the two reaction products.

in the expression site on the 28-kbp plasmid. When the serotype switches from 7 to 21, the copy of the 7 VMP gene in the expression site is removed and replaced by a copy of the 21 VMP gene. The silent copies of the 7 and 21 VMP genes seem unaffected by the switch.

There are three possible mechanisms for the actual recombination reaction: (1) The switch could be the result of a single reciprocal site-specific recombination reaction with subsequent loss of one of the recombination products. (2) The silent genes could lie on 'cassettes' which can be activated by a duplicative transposition. This would then involve a gene conversion event or two cross-overs at either end of the cassette. (3) The silent VMP genes could be activated by insertion of a transposable DNA element upstream of the genes. Model (2) would predict that, in addition to the recombination site that we described, there would be a second recombination site at the other end of the cassette. In model (3) the second site would be at the other end of the inserted transposable element. We have found no such sites. The restriction maps upstream of the expressed 7 and 21 genes are completely colinear for at least 10 kbp, which makes model (3) unlikely. The sequences 3' of the expressed 21 gene (the 2 kbp present in p21.4) show no homology to those 3' of the expressed 7 gene in p7.16, which makes model (2) unlikely. As the plasmids are linear, it is conceivable that the recombination reaction responsible for the switch is a single site-specific cross-over between two linear plasmids. The fact that the approximate distance from the 21 VMP gene to the downstream end of the plasmid is unchanged after the switch is compatible with this idea. On the basis of present evidence, model (1), the single site-specific recombination event, seems the most likely.

The net result of the recombination reaction that fuses a new VMP gene to the expression site will initially be that the cell contains both types of expression plasmids (assuming that only one plasmid switches at a time). For example, in the case described here the plasmid containing the expressed 7 VMP gene could recombine with the plasmid containing the silent 21 gene. The products are the 21 expression plasmid, and another DNA molecule that either segregates out or is actively removed. The cell then contains both type 7 and type 21 expression plasmids. The immune system presumably selects for the segregants that no longer contain the 7 expression plasmid. A prediction following from this model is that cells that express two VMPs at the same time should be detectable *in vitro*. This prediction is now being tested.

Serotype C is different from the serotypes recovered from spirochaetaemic mice. VMP C is much smaller than the VMPs found *in vivo*⁵. No antigenic switching of serotype C cells has

been observed in culture medium, and no relapses have been noted after infection of a mouse with serotype C cells (A.G.B., unpublished observations). As shown, serotype C DNA contains an extra element; it is this DNA segment that contains the expression site for the 7 and 21 VMP genes, and this sequence is fused to yet another sequence in the C strain (Fig. 3A). At present it is unknown whether the sequence is indeed fused to the expressed C VMP gene. The site may be 'empty' and the expressed C gene may be found in another expression site.

We chose to analyse one switch in detail, but it is not necessarily true that the other 24 antigenic types thus far found after infection are all switched on by recombination of the corresponding VMP gene into the same site. A similar analysis to that described here of cell lines of other serotypes will show whether this is the case.

Genetic switches in other organisms

There exist several other examples of DNA rearrangements in prokaryotes that are responsible for switches in gene expression¹³⁻¹⁵. The mechanism of gene activation by recombination of silent genes into an expression site is known in eukaryotes. A binary switch exists in yeast, where the mating type varies by transposition of a silent gene into the *MAT* locus¹⁶. The protozoan *Trypanosoma brucei* can vary its antigens by a mechanism that is very similar to the one described here¹. The parallels between the bacterial and the eukaryotic infectious microorganisms *B. hermsii* and *T. brucei* are striking: the function of the switch (to escape the host immune system), the mechanism of the switch (recombination into an expression site on minichromosomes) and the ordered appearance of the different serotypes are found in both cases^{1,4,5}. It is tempting to speculate on an evolutionary relationship between the two microorganisms. A more likely alternative, however, is that the strong and very direct selective pressure provided by the immune system has resulted in convergence of these two blood-borne and vector-transmitted microorganisms. DNA rearrangements provide the immune system with a means of generating antibodies against a large number of infections; this finds its complement in a system of DNA rearrangements in the microorganism that is designed to escape the immune system.

Linear plasmids and pathogenicity

We have shown that the silent and expressed genes for two surface proteins of *B. hermsii* lie on linear extrachromosomal elements, based on the following evidence. The high frequency with which clones carrying specific VMP genes are found in gene banks shows that these genes must be present many times in the genome. On low-percentage agarose gels and in pulse-field gels, there occur discrete bands of DNA that are not covalently linked to the chromosome. Probes specific for the VMP genes hybridize to these extrachromosomal DNA elements (and not to the chromosome). The DNA molecules carrying the VMP genes have specific ends that are sensitive to degradation by *Bal31* nuclease.

Linear genetic elements have been found previously in prokaryotic cells, but usually they are only transiently linear (for example, several bacteriophages on infection). The DNA of *Bacillus* phage ϕ 29 exists in linear form in the cell and has proteins attached to its ends¹⁷. Linear DNA has been observed in yeast *Streptomyces* sp.¹⁸ and in the mitochondria of a number of Protozoa¹⁹. In *B. hermsii* we detected no circular DNA that hybridizes with the probes specific for the VMP genes, therefore the linear form is probably the replicating form of the plasmid. (Circular plasmid DNA can be isolated from *Borrelia*, but this DNA did not hybridize with the VMP-specific probes; J. Meier and A.G.B., unpublished.) We have demonstrated that after deproteination the DNA molecules are linear, but we do not yet know how the molecules exist in the cell. The fact that only one of the two ends of the molecule seems sensitive to *Bal31* nuclease could mean that there is a covalent modification at one end of the molecule.

Linear plasmids have not been observed previously in pathogenic microorganisms. However, the usual techniques for separating extrachromosomal DNA from the chromosome are largely unsuitable for detecting linear plasmids. On CsCl ethidium bromide gradients, linear extrachromosomal DNA cannot necessarily be separated from the chromosomal DNA. Several other methods for isolation of plasmid DNA allow isolation of only relatively small DNA molecules. There are clear advantages for an infectious microorganism in having a battery of silent and expressed surface protein genes on extrachromosomal DNA: the copy number of the expression plasmid can be high (which facilitates high levels of expression), and

rearrangements can occur at high frequency to activate silent genes, without affecting the stability of the chromosome. An extensive search of other pathogenic microorganisms (and especially those that have a large repertoire of variable antigens) using the technique of pulse-field electrophoresis may reveal an important role of linear extrachromosomal DNA in pathogenesis.

This work was supported in part by grant DMB 820,9295 from the NSF to M.I.S. and by a grant of the Dutch Organization for the Advancement of Pure Scientific Research to R.H.A.P. We thank our colleagues Joe Meier and Sven Bergstrom for useful discussions.

Received 29 July; accepted 11 September 1985.

1. Borst, P. *Mobile Genetic Elements* (ed. Shapiro, J.) 621-659 (Academic, New York, 1983).
2. Hagblom, P., Segal, E., Billgard, E. & So, M. *Nature* **315**, 156-158 (1985).
3. Felsenfeld, O. *Borrelia: Strains, Vectors, Human and Animal Borreliosis* (Green, St Louis, Missouri, 1971).
4. Stoenner, H. G., Dodd, T. & Larsen, C. *J. exp. Med.* **156**, 1297-1309 (1982).
5. Barbour, A. G. & Stoenner, H. G. in *Genome Rearrangements* (eds Herskowitz, I. & Simon, M.) (Liss, New York, 1985).
6. Kelly, R. *Science* **173**, 443-444 (1971).
7. Barbour, A. G., Tessier, S. L. & Stoenner, H. G. *J. exp. Med.* **156**, 1312-1316 (1982).
8. Barbour, A. G., Barrera, O. & Judd, R. C. *J. exp. Med.* **158**, 2127-2140 (1983).
9. Barstad, P. A., Coligan, J. E., Raum, M. G. & Barbour, A. G. *J. exp. Med.* **161**, 1302-1314 (1985).
10. Meier, J. T., Simon, M. I. & Barbour, A. G. *Cell* **41**, 403-409 (1985).
11. Frischauf, A. M., Lehrach, H., Poustka, A. & Murray, N. *J. molec. Biol.* **170**, 827-842 (1983).
12. Schwartz, D. C. & Cantor, C. R. *Cell* **37**, 67-75 (1984).
13. Zieg, J. & Simon, M. I. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4196-4200 (1980).
14. Van de Putte, P., Cramer, S. & Giphart-Gassler, M. *Nature* **286**, 218-222 (1980).
15. Golden, J. W., Robinson, S. J. & Haselkorn, R. *Nature* **314**, 419-423 (1985).
16. Nasmyth, K. A. *A. Rev. Genet.* **16**, 439-500 (1982).
17. Watabe, K., Shih, M.-F., Sugino, A. & Ito, J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 5245-5248 (1982).
18. Hiroshika, H. & Sakaguchi, K. *Plasmid* **7**, 59-65 (1982).
19. Goldbach, R. W., Bollen-de Boer, J. E., Van Bruggen, E. F. J. & Borst, P. *Biochim. biophys. Acta* **562**, 400-417 (1979).

A cDNA clone encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*

J. B. Nasrallah*, T.-H. Kao†, M. L. Goldberg‡ & M. E. Nasrallah*

* Sections of Plant Biology, † Biochemistry, Molecular and Cell Biology and ‡ Genetics and Development, Cornell University, Ithaca, New York 14853, USA

The pollen-stigma interaction resulting in self-incompatibility in the cruciferous plant Brassica has been correlated with glycoproteins that display polymorphisms in all S genotypes examined. A complementary DNA clone encoding these glycoproteins has been isolated and characterized. Polymorphisms in homologous restriction fragments of Brassica genomic DNA segregate precisely with alleles at the S locus.

IN many species representing very diverse angiosperm families, self-fertilization is blocked by a mechanism termed self-incompatibility¹. Following self-pollination, interaction between the stigma or style and pollen elicits a defined morphological response preventing normal pollen tube growth; fertilization is prevented because these pollen tubes are unable to penetrate the full length of the stigma and style. The specificity of the incompatible interaction is determined by one or more genetic loci, and requires that the alleles carried by the male and female parents are identical. In the crucifer *Brassica*, the self-incompatible reaction is localized at the stigma surface, and occurs within minutes after the initial contact between the pollen and the papillar cells on the outer surface of the stigma. In this genus, self-incompatibility is under the control of a single genetic locus, the *S* locus, which is highly polymorphic, some 50 alleles having been identified^{2,3}.

A molecular analysis of the genetic control of incompatibility may best be performed by detecting antigens specific to various *S*-locus alleles in stigma homogenates from different *Brassica* strains⁴. These antigens have been shown to correspond to glycoproteins that may be resolved in various electrophoretic systems⁵⁻⁸. Several lines of evidence suggest that these glycoproteins have an important role in incompatibility. (1) The mobilities of these molecules vary in stigma extracts derived from *Brassica* strains with different *S*-locus alleles⁸. (2) These molecules are found in the stigma but not in style or seedling tissue⁹. (3) The increased rate of synthesis of these *S*-locus-specific glycoproteins (SLSGs) in the developing stigma correlates with the onset of the incompatibility reaction in the stigma⁹. (4) Mutations in genes unlinked to the *S* locus which result in self-compatibility are also associated with reduced levels of these

molecules¹⁰. (5) Most importantly, the inheritance of the various forms of SLSG correlates perfectly with the segregation of *S* alleles in genetic crosses, indicating that the gene responsible for this polymorphism must be genetically located at or extremely closely linked to the *S* locus^{6,11}.

Here we report the isolation of a complementary DNA clone containing sequences encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*. We show that the spatial and temporal distribution of the messenger RNA homologous to these sequences mirrors the appearance of the *S*-locus-specific glycoprotein. Several fragments of *B. oleracea* genomic DNA, generated by restriction endonucleases, hybridize with the SLSG cDNA clone; polymorphisms in certain of these fragments segregate precisely with alleles at the *S* locus.

Isolation

Our strategy for isolating SLSG-encoding DNA sequences was based on the finding that, in some *Brassica* strains, ~5% of the protein synthesis in the self-incompatible bud stigma is devoted to the SLSG synthesis⁹. Thus, it seemed likely that a high proportion of clones in a cDNA library constructed with poly(A)⁺ RNA from mature, incompatible stigmas would contain SLSG sequences. It had also been observed previously that SLSG molecules appear in the stigma but not in leaf or seedling tissue⁸. We thus expected that in such a library SLSG cDNA clones would be enriched in the fraction of clones that would hybridize strongly to radioactive cDNA¹² corresponding to mature stigma mRNA, but not to labelled leaf or seedling-specific cDNA. Poly(A)⁺ RNA from incompatible stigmas was therefore isolated and used as a template for a cDNA library constructed in the plasmid pBR322 using *Bam*HI linkers¹³.

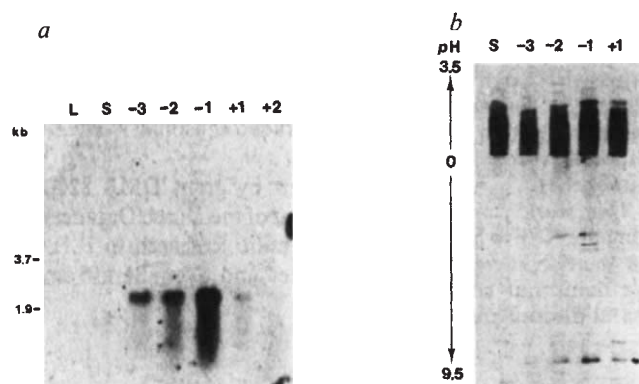


Fig. 1 Temporal regulation of pBOSS5-specific RNA and of SLSG expression in the S_6S_6 homozygote. *a*, Northern analysis of RNA from *Brassica* tissues. L, leaf tissue; S, stylar tissue; -3, -2, -1, +1 and +2 refer to stigma developmental stages in days before or after anthesis. *b*, Synthesis of SLSG in developing stigmas. The origin of isoelectric focusing 'O' on the autoradiograph and the generated pH gradient are indicated. The arrow indicates the S_6 -specific SLSG band. Lane designations as in *a*.

Methods. Each inflorescence was divided into developmental zones designated -3, -2, -1, +1 and +2, each consisting of a sequence of three flowers or flower buds. Stigmas from the -3, -2 and -1 zones were stigmas derived from immature flower buds that would normally reach anthesis in 3, 2 and 1 days, respectively; stigmas from the +1 and the +2 zones were mature stigmas from flowers at 1 and 2 days past anthesis, respectively. Stigmas from the -3 and -2 zones are self-compatible, while those from the remaining zones are self-incompatible. *a*, RNA was isolated from *Brassica* tissues using an SDS-proteinase K extraction procedure^{21,22}. Samples containing 1 μ g of denatured RNA were electrophoresed on 1% (w/v) agarose gels in the presence of formaldehyde¹³ and transferred to nitrocellulose²³. Parallel untransferred lanes were stained with ethidium bromide and the location of the ribosomal RNA bands determined. Blots were prehybridized at 42 °C in 50% formamide (w/v), 1% Denhardt's solution, 0.2% SDS (w/v), 0.9 M NaCl, 50 mM NaH_2PO_4 pH 7.4, 5 mM EDTA and 100 μ g ml^{-1} carrier calf thymus DNA. The filters were hybridized to nick-translated pBOSS5 DNA²⁴, washed and exposed to Kodak XAR-5 film. *b*, Excised stigmas and 1-mm sections of stylar tissue were metabolically labelled by submersion in a ^{14}C -amino-acid mixture as described elsewhere⁹. Isoelectric focusing analysis of radioactively labelled proteins and autoradiography were also as previously described⁹.

Approximately 2,000 independent clones were picked randomly from this cDNA library, and DNA from these colonies was then transferred to each of two nitrocellulose filters and hybridized with the appropriate probes¹⁴. Eight colonies were found to hybridize strongly with stigma-derived cDNA probe but not with seedling cDNA probe. Plasmid DNA was prepared from each of these eight colonies, and digests with the restriction endonuclease *Bam*HI were performed such that the cDNA inserts could be separated from the plasmid vector by gel electrophoresis. Cross-hybridization experiments performed on these digests indicated that the *Brassica* DNAs in all eight recombinant plasmids were homologous (results not shown). The plasmid containing the largest cDNA insertion, pBOSS5, was chosen for use in all subsequent experiments reported here.

pBOSS5 expression during development

To examine more directly the proposition that the inserts in plasmid pBOSS5 and its relatives correspond to SLSG mRNA, the concentration of pBOSS5-homologous mRNA was measured as a function of stigma development (Fig. 1). Northern analysis of RNA prepared from populations of staged stigmas reveals a prominent band ~2 kilobases (kb) long which cannot be detected in leaf and style tissue (Fig. 1a). This RNA species must be quite abundant in the stigma, as intense signals are obtained even when poly(A)⁺ RNA has not been purified. The pattern of expression of these sequences parallels the pattern of synthesis

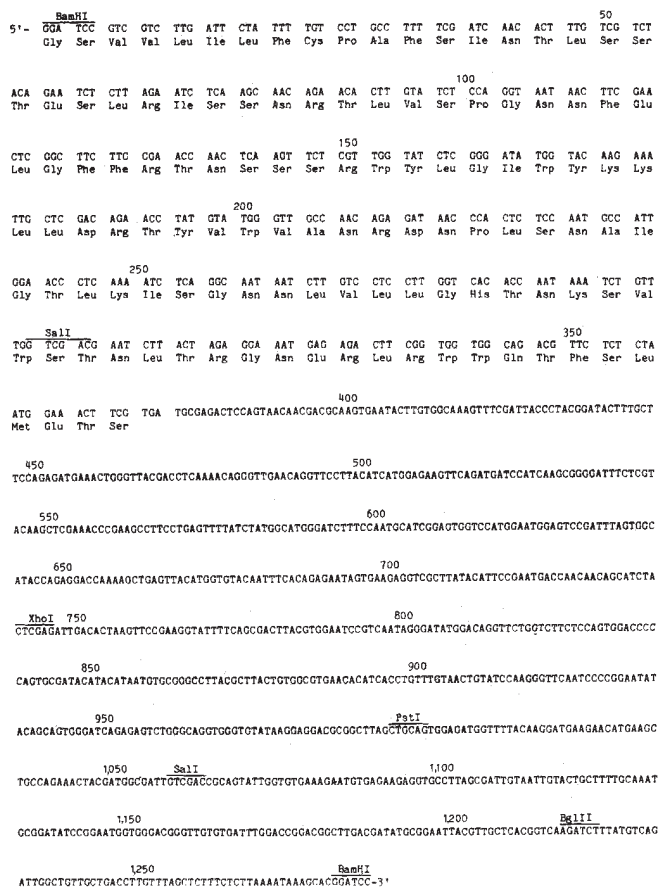


Fig. 2 Nucleotide sequence and deduced amino-acid sequence of the cDNA insert in pBOSS5. Sequences delineated by the indicated restriction sites were subcloned into the M13 vectors M13 mp10 and M13 mp11 (ref. 25). Sequencing was carried out by the dideoxynucleotide chain-termination method¹⁵ using a synthetic 17-mer as primer (New England Biolabs).

of SLSG in the stigma (Fig. 1b), a maximum level being observed 1 day before the flower opens. It should be emphasized that the stigmas of immature buds are self-compatible, so that fertilization with pollen which would normally induce an incompatible response is possible by mechanically opening the bud and placing such pollen directly on the stigma. As the buds develop, the stigmas become self-incompatible 1 day before anthesis, correlating with the observed peak concentration of pBOSS5-homologous RNA.

An S-locus-specific glycoprotein

Although the previous results were consistent with the notion that pBOSS5 contains sequences encoding SLSG, further tests were required to confirm the identity of this clone. Thus, the nucleotide sequence of the cDNA insert in pBOSS5 was determined to guide strategies for the construction of chimaeric genes in which *Brassica* DNA could be transcribed and translated in *Escherichia coli* cells, in order to establish whether the resultant fusion protein would react with antiserum to authentic SLSG. Restriction endonuclease-generated fragments of the pBOSS5 insert were subcloned into the M13 vectors mp10 and mp11 and the resulting single-stranded DNAs subjected to sequence analysis by the Sanger technique¹⁵.

Figure 2 shows the results of the nucleotide sequence determination. Although five of the six possible reading frames are blocked by multiple nonsense codons throughout the entire 1,284-base pair (bp) insert, the remaining reading frame translates into a polypeptide of 123 amino acids. The nucleotide sequence AATAAA, which has been implicated as a signal for the polyadenylation of mRNA¹⁶, is located downstream of the putative SLSG coding region, 11–16 bp from the end of the

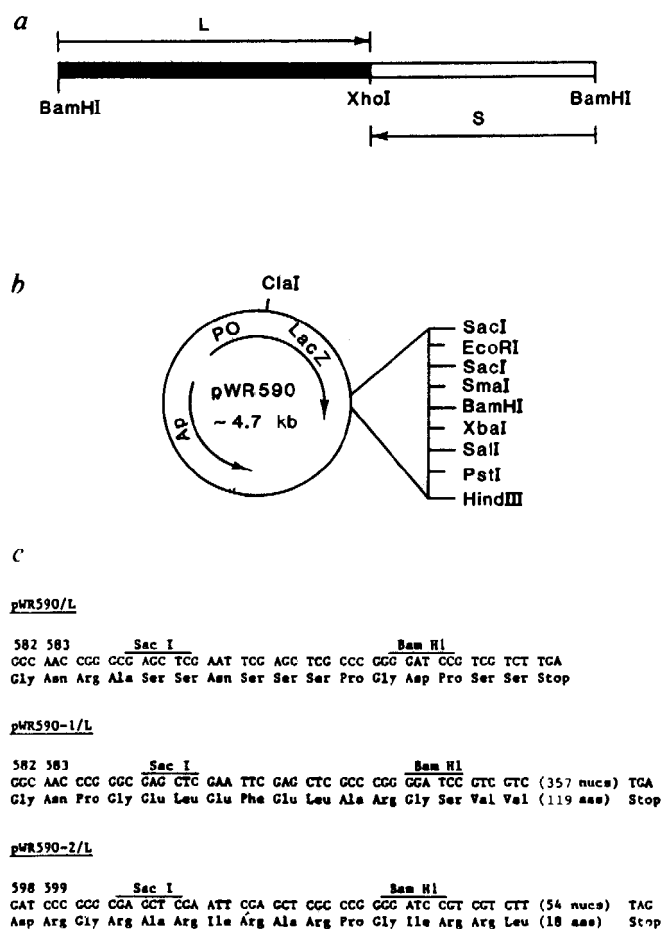


Fig. 3 Subcloning of the pBOS5 cDNA insert into the pWR590 series of expression vectors. **a**, Generation of the pBOS5 L and S fragments by *Bam*HI/*Xho*I digestion. Arrows indicate the resulting direction of transcription in the chimaeric plasmid. **b**, Map of pWR590. Arrows indicate the direction of transcription. *LacZ'* denotes a shortened *lacZ* gene encoding about 590 amino acids. The expanded region following the *lacZ'* gene contains the poly-linker sequence. **c**, Nucleotide sequences of the expression vectors at the fusion junctions, and the expected translational products. nucs, nucleotides; aas, amino acids.

Methods. To construct expression plasmids of the *Brassica* cDNA sequence, the 745-bp (L) and 538-bp (S) *Bam*HI/*Xho*I fragments of pBOS5 were isolated from a 1% low-melting agarose gel and ligated to *Bam*HI and *Sall* digests of pWR590, pWR590-1 and pWR590-2. The resulting six plasmid constructions, designated pWR590-L, pWR590-1L, pWR590-2L, pWR590-S, pWR590-1S and pWR590-2S, were used to transform *E. coli* SF8 cells²⁶.

cDNA insert presumed to have been derived from the 3' end of the mRNA. Such an interpretation of the sequence data implies that the 3'-noncoding region of the messenger is ~900 nucleotides long. Although such an extensive 3'-noncoding region is unusual, precedents have been noted in other eukaryotic mRNAs¹⁷. The absence of a poly(A) tract may be explained by the finding that the *Bam*HI sequences flanking both sides of the cDNA insert do not resemble the *Bam*HI linker sequence used to construct the cDNA clone. We presume that a *Bam*HI recognition site is located in the full-length cDNA sequence between the polyadenylation signal and the poly(A) tail of the messenger, such that this *Bam*HI site would be inserted into the pBR322 vector in place of the end provided by the linker sequence. Our inability to obtain clones with longer cDNA sequences is similarly explained by the presence of at least one additional *Bam*HI site in sequences internal to the mRNA.

Although incomplete, the predicted polypeptide has properties consistent with its corresponding to SLSG. Approximately 11% of the amino acids are basic and only 5% are acidic, in

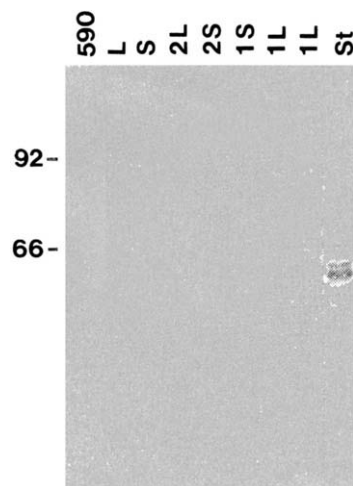


Fig. 4 Western blot analysis of fusion proteins encoded by constructs in the pWR590 series of expression vectors. 'St' designates the lane with a stigma extract; the characteristic M_r heterogeneity⁸ of SLSG is shown. Designations above the other lanes indicate extracts from *E. coli* cells transformed with the corresponding pWR590 constructs. One band corresponding to the antigenic fusion protein is seen in lanes 1L. The mobilities of M_r standards are shown ($\times 10^{-3}$).

Methods. Transformed *E. coli* SF8 strains were grown overnight in the presence of 1 mM isopropyl thiogalactoside. Extracts were prepared by sonication in 7 M guanidine hydrochloride¹⁹, and aliquots equivalent to 500 μ l of culture were subjected to electrophoresis on 10% SDS acrylamide gels²⁷. The separated proteins were transferred electrophoretically to nitrocellulose membrane²⁸. Immunolabelling of nitrocellulose blots was as described elsewhere²⁹. The first-stage antiserum was raised in a New Zealand White rabbit by multiple intradermal injections of purified S₆-specific glycoprotein. The second-stage probe was peroxidase-conjugated protein A.

keeping with the basic nature of most SLSG molecules analysed to date. Four potential *N*-glycosylation sites which follow the general rule of Asn-X-Thr/Ser (ref. 18) can be identified beginning at asparagine residues 28, 46, 96 and 103. Many serine and threonine residues are also potentially available for *O*-glycosylation.

The presumptive coding region of the cDNA insert is entirely contained within a fragment of DNA (fragment L in Fig. 3a) demarcated by recognition sites for the enzymes *Bam*HI (at positions 1-6) and *Xho*I (nucleotides 740-745). A family of three expression vectors (pWR590, pWR590-1 and pWR590-2) has recently been described in which foreign DNA may be inserted in all three possible translational reading frames into a polylinker region such that these exogenous sequences can be translated by *E. coli* cells into a fusion protein, the first 590 amino acids of which are supplied by a truncated *E. coli* β -galactosidase gene (ref. 19 and Fig. 3b). As illustrated in Fig. 3c, fragment L from pBOS5 theoretically would encode a fusion protein containing determinants encoded by the long open reading frame predicted by the DNA sequence data only when inserted into the vector pWR590-1.

Fragment L and fragment S, a *Bam*HI/*Xho*I fragment containing the remainder of the *Brassica* insert (Fig. 3a), were cloned into the pWR590 vectors such that all six possible reading frames of the *Brassica* cDNA could be assayed for their ability to promote the expression in *E. coli* cells of polypeptide determinants which could react with antiserum to authentic SLSG glycoprotein. Extracts prepared from cultures of *E. coli* transformed with the six constructs were probed with antibodies to SLSG (Fig. 4). It may be seen that in lanes 1L (pWR590-1L, see Fig. 3c), a band of relative molecular mass (M_r) ~85,000 reacts with the antiserum. As predicted, this band is not found in lysates from cells containing any of the other five chimaeric plasmids or the parental pWR590 vector. Furthermore, the size

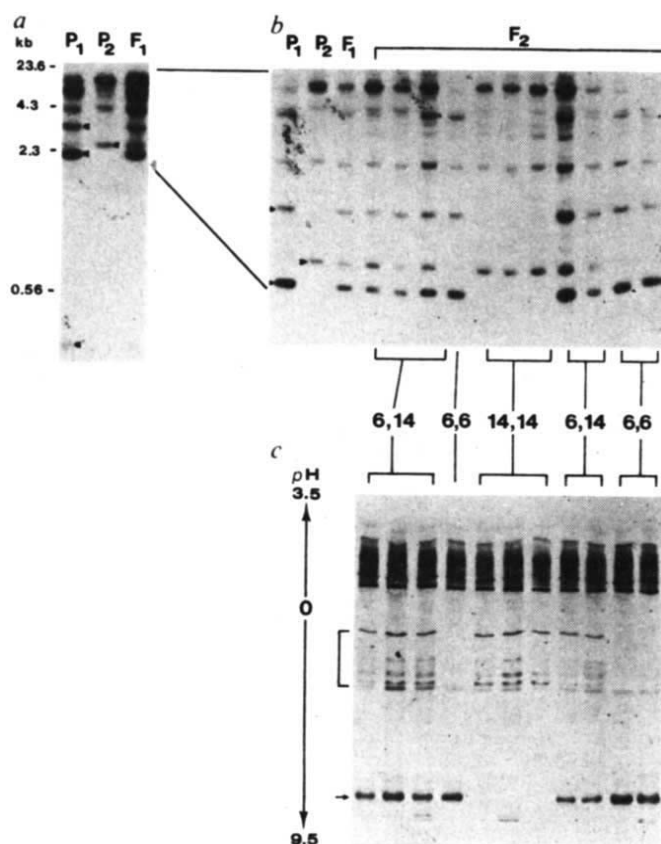


Fig. 5 Analysis of a population of plants segregating for the S_6 and S_{14} alleles. *a*, Southern analysis of *Bam*HI/*Pst*I digests of genomic DNA from the S_6 -homozygous parent (P_1), the S_{14} -homozygous parent (P_2) and the heterozygous F_1 hybrid. Arrowheads indicate representative restriction fragments that segregate in correlation with the S_6 and S_{14} alleles. The nick-translated cDNA insert of pBOS5 was the probe. *b*, Southern analysis of *Bam*HI/*Pst*I digests of DNA from P_1 , P_2 , F_1 and an F_2 population derived by self-pollinating immature self-compatible stigmas of the F_1 heterozygotes. Electrophoresis was carried out longer than in *a* in order to resolve the higher- M_r restriction fragments. 6,6: S_6 homozygotes; 14,14: S_{14} homozygotes; 6,14: S_6S_{14} heterozygotes. The probe and arrowheads are as in *a*. *c*, Isoelectric focusing patterns of stigma extracts from the F_2 plants in *b*. The arrow indicates the S_6 -specific band and the bracket indicates the complex of bands characteristic of the S_{14} allele. The origin of electrophoresis (O) and the pH gradient are indicated. The gel was stained with Coomassie blue R250.

Methods. The S_6 and S_{14} homozygotes are inbreds maintained at Cornell. Incompatibility phenotypes were determined in F_2 plants by crossing to tester homozygous strains and by diallel analysis. The pollinated flower stigmas were stained with decolorized aniline blue and pollen-tube development monitored by fluorescence microscopy^{30,31}. DNA was prepared from ~0.25 g of leaf tissue by a modification of a rapid phage lysis protocol. Digests of genomic DNA were electrophoresed on 0.9% (w/v) agarose gels and transferred to GeneScreen filters (NEN)³². Filters were prehybridized and hybridized in 50% (w/v) dextran sulphate, 330 mM NaP_i, pH 7.0, 10 mM EDTA, 5% (w/v) SDS and 100 μ g ml⁻¹ carrier salmon testes DNA, at 67 °C. Washed filters were exposed to Kodak XAR-5.

of the prominent band is that expected for a fusion protein consisting of 593 amino acids from β -galactosidase and the polylinker region and 123 amino acids encoded by the *Brassica* cDNA clone.

Genomic pattern

Southern analysis of *Brassica* genomic DNA cleaved with a variety of restriction enzymes, including *Eco*RI, *Sal*I, *Xho*I, *Bam*HI and *Pst*I, revealed *S*-allele-associated restriction site polymorphisms. In each digest, several bands hybridized with

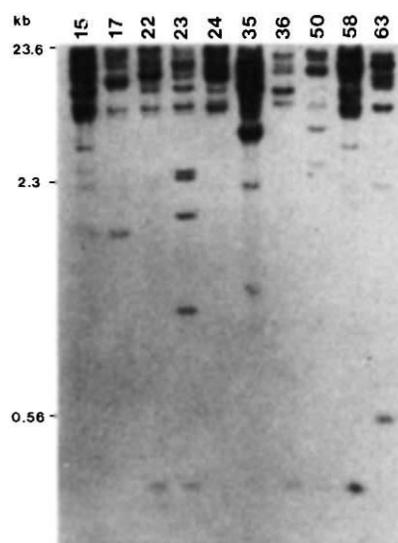


Fig. 6 Southern analysis of *Bam*HI/*Pst*I digests of DNA from different *S*-allele homozygotes of *Brassica*. The homozygous lines were obtained from the Gene Bank Facility at Wellesbourne, Warwick, UK, courtesy of Dr David Ockendon. The numbers above the lanes refer to the *S*-allele designations of the Gene Bank Facility. The probe is as in Fig. 5.

pBOS5. To determine whether these polymorphisms segregate with the *S* locus, DNA was prepared from an F_2 population obtained by the selfing of heterozygous F_1 plants derived from S_6S_6 and $S_{14}S_{14}$ homozygotes. Analysis of this DNA cleaved with *Bam*HI and *Pst*I showed that some pBOS5-homologous bands are common to all of the plants examined, but that others are clearly specific to the S_6 or S_{14} alleles (arrowheads in Fig. 5*a, b*). Heterozygous S_6S_{14} plants display a hybrid pattern. Similar results have been observed for 55 plants from this F_2 cross and for an additional 22 F_2 plants segregating for the S_6 and S_{13} alleles. In all cases, the restriction site polymorphisms revealed by the pBOS5 probe co-segregate with the *S*-locus genotype as determined by pollination analysis, and with the SLSG polymorphism as determined by isoelectric focusing. A prominent band with an isoelectric point of ~9.0 is present in S_6S_6 homozygotes (Fig. 5*c*), but is replaced by a series of bands only slightly basic in charge in homozygous $S_{14}S_{14}$ plants. Of the heterozygous plants examined, all synthesize both sets of SLSG molecules.

Finally, the restriction endonuclease-generated fragments of *Brassica* DNA homologous to pBOS5 in plants homozygous for each of 14 different *S*-locus alleles demonstrate patterns specific to each genotype (Fig. 6) and suggest a variability sufficient to account for the 50 or so *S*-locus alleles known in *Brassica* populations.

Discussion

Our results show that pBOS5, and presumably the seven other homologous cDNA clones we have isolated, contain sequences specifying an *S*-locus-specific glycoprotein (SLSG). This identity has been established directly by the demonstration that pBOS5 encodes SLSG-specific antigenic determinants. A chimaeric gene, in which the cDNA insert from pBOS5 is joined in-frame with the *lacZ* gene from *E. coli*, directs the synthesis in *E. coli* cells of a fusion polypeptide which reacts with anti-serum against authentic SLSG. Furthermore, the characteristics of the mRNA from which pBOS5 was derived are consistent with a molecule capable of encoding SLSG. This mRNA species is found in stigma tissue, but cannot be detected by our methods in styles, leaf or seedling tissue. The concentration of this messenger varies as a function of the maturity of the stigma in parallel with the rate at which SLSG is synthesized. Finally, DNA sequencing studies suggest that the 2-kb RNA homologous with pBOS5 is of sufficient length to encode SLSG. Although

estimates of the relative molecular mass of SLSG are complicated by the fact that these macromolecules are heavily glycosylated²⁰, preliminary experiments using material in which the carbohydrate moieties have been chemically removed from SLSG suggest M_r of 40,000–50,000 (J.B.N., unpublished results). Accounting for the long 3'-noncoding region deduced from the sequence data, over 1,100 nucleotides of protein-coding information remains in the 2-kb mRNA, sufficient to encode a polypeptide of 45,000 M_r . Furthermore, the amino-acid composition of the predicted polypeptide is consistent with that reported for three SLSGs in *B. oleracea*²⁰.

As outlined above, several lines of evidence strongly suggest that SLSG is the product of the *S* locus. The clearest support is provided by the finding that polymorphisms in the SLSG molecule itself and restriction site polymorphisms in the vicinity of certain genomic DNA sequences homologous with pBOSS co-segregate with *S*-locus alleles. DNA likely to encode SLSG is thus genetically at or very closely linked to the *S* locus. Even in the event of fortuitous linkage between SLSG-encoding sequences and *S*-locus sequences, the latter would therefore be accessible to molecular cloning by the use of chromosome walking procedures extending from pBOSS-related genomic fragments.

Note that only the subset of the genomic restriction fragments homologous with pBOSS which are specific for the *S*-locus genotype can be shown to segregate with the *S* alleles in the analysis reported here. Thus, these experiments do not completely rule out the possibility that the gene for SLSG is located on a segment of DNA unlinked to the *S* locus. If this is so, the SLSG polypeptide specified by this unlinked segment must be modified in some manner by a different molecular product of the *S* locus. However, the bands which exhibit polymorphisms are, in general, those which hybridize most strongly to the pBOSS probe, and are thus most likely to have served as the template for the transcription of SLSG message.

At present, the simplest hypothesis consistent with the data asserts that SLSGs are in fact the products of the *S* locus. This assumption may be tested directly by a future demonstration that the transformation of plants with cloned genomic DNA selected with pBOSS can alter their incompatibility specificity.

We thank L. H. Guo and R. Wu for providing expression vectors pWR590, pWR590-1 and pWR590-2 before publication, and R. Wu and A. M. Srb for the use of their laboratory facilities in carrying out part of our work. This work was supported by a grant from the Cornell Biotechnology Program to J.B.N. and M.L.G., and by NSF grant PCM8118035 to M.E.N.

Received 15 July; accepted 17 September 1985.

- East, E. M. *Proc. Am. phil. Soc.* **82**, 449–518 (1940).
- Thompson, K. F. & Taylor, J. P. *Heredity* **21**, 345–362 (1966).
- Ockendon, D. J. *Heredity* **33**, 159–171 (1974).
- Nasrallah, M. E. & Wallace, D. H. *Heredity* **22**, 519–527 (1967).
- Nasrallah, M. E., Barber, J. T. & Wallace, D. H. *Heredity* **25**, 23–27 (1970).
- Nasrallah, M. E., Wallace, D. H. & Savo, R. M. *Genet. Res.* **20**, 151–160 (1972).
- Nishio, T. & Hinata, K. *Heredity* **38**, 391–396 (1977).
- Nasrallah, J. B. & Nasrallah, M. E. *Experientia* **40**, 279–281 (1984).
- Nasrallah, J. B., Doney, R. C. & Nasrallah, M. E. *Planta* **165**, 100–107 (1985).
- Nasrallah, M. E. *Genetics* **76**, 45–50 (1974).
- Hinata, K. & Nishio, T. *Heredity* **41**, 93–100 (1978).
- Taylor, J. M., Illmensee, R. & Summers, J. *Biochim. biophys. Acta* **442**, 324–330 (1976).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961–3965 (1975).
- Sanger, F., Coulson, A. R., Barrell, B. G., Smith, A. J. H. & Rose, B. J. *molec. Biol.* **143**, 161–178 (1980).
- Proudfoot, N. J. & Brownlee, G. G. *Nature* **263**, 211–214 (1976).
- Ullrich, A. *et al.* *Nature* **309**, 418–425 (1984).

- Neuberger, A., Gottschalk, A., Marshall, R. D. & Spiro, R. G. in *The Glycoproteins: Their Composition, Structure and Function* Pt A (ed. Gottschalk, A.) 450–490 (Elsevier, Amsterdam, 1972).
- Guo, L. H. *et al.* *Gene* **29**, 251–254 (1984).
- Nishio, T. & Hinata, K. *Genetics* **100**, 644–647 (1982).
- Hall, T. C. *et al.* in *Genome Organization and Expression in Higher Plants* (ed. Leaver, C.) 259–272 (Plenum, New York, 1980).
- Beachy, R. N., Barton, K. A., Madison, J. T., Thompson, J. F. & Jarvis, N. in *Genome Organization and Expression in Higher Plants* (ed. Leaver, C.) 273–281 (Plenum, New York, 1980).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201–5205 (1980).
- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. J. *molec. Biol.* **113**, 237–251 (1977).
- Messing, J. & Vieira, J. *Gene* **19**, 269–276 (1982).
- Hanahan, D. J. *molec. Biol.* **166**, 557–580 (1983).
- Laemmli, U. K. *Nature* **227**, 680–685 (1970).
- Towbin, H., Staehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).
- Symington, J. in *Two-Dimensional Electrophoresis of Proteins* (eds Celis, J. & Bravo, R.) 129–168 (Academic, New York, 1983).
- Linskens, H. F. & Esser, K. L. *Naturwissenschaften* **44**, 1–2 (1957).
- Martin, F. W. *Stain Techn.* **34**, 125–128 (1959).
- Southern, E. J. *molec. Biol.* **98**, 503–517 (1975).

LETTERS TO NATURE

A cosmic-ray explanation of the galactic ridge of cosmic X-rays

C. L. Bhat*, T. Kifune* & A. W. Wolfendale

Physics Department, University of Durham, South Road, Durham DH1 3LE, UK

Recent observations¹ with the Exosat X-ray satellite have confirmed the existence of an apparently diffuse galactic ridge of emission extending over the inner Galaxy ($l = 320^\circ$ – 40°) and confined to about $\pm 2^\circ$ in galactic latitude. On the basis of measurements² carried out in the galactic plane at $l \approx 55^\circ$, we have proposed previously³ that the diffuse galactic X-ray flux known at that time could be understood in terms of the synchrotron radiation of cosmic-ray electrons in the galactic magnetic field. The more extensive Exosat measurements fit nicely into this picture and the X-ray-producing electrons can now be readily understood as being secondary to the ultra-high-energy protons, which are themselves generated in galactic objects as epitomized by the recently discovered^{4,5} Cygnus X-3. We show here that, on this model, the number of Cyg X-3-like objects present in the Galaxy at any one time, averaged over the past 10^3 yr or so, is ≈ 30 .

The first report of an apparently diffuse X-ray flux from the galactic plane seems to have been by Cooke *et al.*⁶ in 1969. The most recent¹ work, using Exosat for two-dimensional mapping, strongly supports the existence of the ridge, and Warwick *et al.*¹ estimate an overall galactic diffuse emission of $\approx 1 \times 10^{38}$ erg s⁻¹ in the energy range 2–6 keV.

Figure 1 reproduces some of the Exosat results, together with a profile of the synchrotron emission at 408 MHz from ref. 7. The bulk of the 408-MHz radiation is due to GeV cosmic-ray electrons interacting with the galactic magnetic field. The similarity in the X-ray emission distribution with the synchrotron radiation profile is encouraging for our model but not conclusive, of course, because of the significant differences in spatial distributions of the X-ray-producing electrons ($\sim 10^{13}$ – 10^{15} eV) and the GeV electrons responsible for the 408-MHz radiation; the z (height above the galactic plane) distributions are very different.

The normalization in Fig. 1 is made at the minima in the observed X-ray profile in the region of $l \approx 30^\circ$ and 330° , because these low values should more closely represent the genuinely diffuse X-ray component between the sources (we consider that the minima at $|l| < 30^\circ$ are contaminated by contributions from unresolved discrete sources). The corresponding total X-ray emission is lower by a factor of ~ 2 than the estimate made by Warwick *et al.*¹ and is thus $\sim 5 \times 10^{37}$ erg s⁻¹. Another difference between our approach and that of Warwick *et al.*¹ is that we believe that the 'dips' in the X-ray profile, rather than reflecting

Permanent addresses: *Bhabha Atomic Research Centre, Nuclear Research Laboratory, Sinagar, Kashmir, India (C.L.B.); Institute for Cosmic ray Research, University of Tokyo, Tanashi, Tokyo, Japan (T.K.).

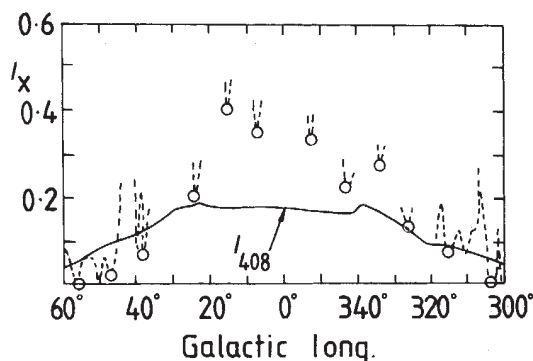


Fig. 1 Longitude distribution of the 'diffuse' X-ray emission, in the range 2–6 keV, from ref. 1 (Exosat data); the dashed line represents the profile and the circles the minima. The units are $7 \times 10^{-11} \text{ erg s}^{-1} \text{ cm}^{-2}$ per beamwidth. Also shown is the 'model' that we adopted based on the 408-MHz radio profile at $b = 0^\circ$ (ref. 7), normalized to the X-ray flux minima at $l \sim 30^\circ$ and 330° . The patchiness in the observed X-ray emission probably comes from both the presence of unresolved discrete sources, which are difficult to allow for, and the fact that the ultra-high-energy electrons, producing the synchrotron X rays, have only a limited diffusion away from their sources during the average electron lifetime of $\approx 2 \times 10^3 \text{ yr}$ against radiative losses.

excessive absorption, correspond to regions of lower intrinsic emission as well as inter-source regions.

The magnitude of the absolute electron flux that is compatible with the observed X-ray emission is of the order of $\sim 10^{-24} \text{ cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ eV}^{-1}$ at 10^{14} eV , as inferred by Protheroe and Wolfendale⁸ to explain the then known diffuse X-ray flux^{2,3}. This electron flux corresponds to an injection spectrum having a roughly constant exponent from much lower energies and it is higher than that based on an extrapolation of the local electron spectrum, known only up to particle energies of $\sim 10^{12} \text{ eV}$. The argument advanced then⁸ to account for this apparent disparity was that we are not near enough to an ultra-high-energy electron source, at present, to measure the actual average galactic intensity, which was admittedly a rather *ad hoc* justification.

The new development that has strengthened the above argument, in particular for a significant contribution to the galactic X-ray ridge coming from the cosmic ray electrons, has been the discovery^{4,5} of ultra-high-energy γ rays, up to 10^{16} eV , from Cyg X-3, a binary system about 12 kpc from the sun. The γ rays are probably^{9,10} secondary to the protons which are first accelerated in the electromagnetic fields of one of the binary components (neutron star?) and then interact with the gas present in the atmosphere of the companion star. As a result, there will be electrons associated with the $\pi^+ \rightarrow \mu^+ \rightarrow e^+$ cascades corresponding to γ rays from the alternative π^0 -decay chain. The ratio of electrons to γ rays cannot be calculated accurately because of insufficient knowledge about the production region, but it is unlikely to be far from the factor 0.5 expected on the basis of kinematics, and we adopt this value here. We assume that the electrons can escape from the 'source region' and are not trapped by magnetic fields, a reasonable assumption in that the parent protons must have had near-rectilinear motion to preserve the γ -ray phase.

Concerning the energetics of the process, $\gamma \rightarrow e \rightarrow X$ rays, we note that the γ -ray energy per decade from Cyg X-3 (1978 level⁴) is $\sim 10^{37} \text{ erg s}^{-1}$, centred on $5 \times 10^{14} \text{ eV}$, the energy required to give X rays in the middle of the detected range (2–6 keV). The X-ray energy is proportional to the square of the electron energy so that the progenitor-electrons will have energy $E_e \sim (4\text{--}7) \times 10^{14} \text{ eV}$, corresponding to the Cyg X-3 output of $0.5 \times 3 \times 10^{36} \text{ erg s}^{-1}$, that is, $1.5 \times 10^{36} \text{ erg s}^{-1}$.

If, as is likely, Cyg X-3-type sources represent a typical galactic population, such as pulsars, supernova remnants and

H II regions, and have a spatial distribution similar to that of the GeV cosmic-ray electrons¹¹ responsible for the radio synchrotron emission—a peak intensity of about twice the local value at a galactocentric distance $R = 6 \text{ kpc}$ —then the longitude distribution will be similar to that denoted by I_{408} in Fig. 1.

As was pointed out earlier, the total energy requirement in electrons is $\sim 5 \times 10^{37} \text{ erg s}^{-1}$ to account for the Exosat X-ray ridge so that there should be ~ 30 sources contributing, each emitting $\sim 1.5 \times 10^{36} \text{ erg s}^{-1}$ for $E_e \sim (4\text{--}7) \times 10^{14} \text{ eV}$. Note that this is not necessarily the number 'switched on' at present because the mean lifetime of the electrons due to the synchrotron losses in the galactic magnetic field is $\sim 2 \times 10^3 \text{ yr}$ at $E_e \sim 5 \times 10^{14} \text{ eV}$. Thus, this number corresponds to the average value over a time of this order.

The number appears to be large but is not implausible, as is evident from the following observations. (1) There is a suggestion¹² that Cyg X-3 has been considerably stronger in the past and the ultra-high-energy photon flux ($\sim 10^{15} \text{ eV}$) may have decreased by a factor as large as ~ 80 in the past decade only¹³, for which the data are available. (2) A source (LMC X-4), stronger than Cyg X-3 by a factor ~ 20 , has been reported¹⁴ in the Large Magellanic Cloud. (3) Cosmic-ray anisotropy measurements at $> 10^{15} \text{ eV}$ suggest that Cyg X-3 is not unique and that there may be up to 10 similar sources in the Galaxy at present¹⁵. Similarly, at lower energies, between 1 and $3 \times 10^{13} \text{ eV}$, the recent anisotropy measurements¹⁶ at Baksan Valley and Norikura indicate¹⁷ an 'excess' near Right Ascension (RA) $\sim 300^\circ$ with respect to the general flow of particles from RA: $40^\circ\text{--}100^\circ$. If interpreted as due to γ rays, this excess leads to an upper bound of ~ 70 on the number of sources operating in the Galaxy at present (see ref. 17). (4) A recent analysis of muon-poor showers by the Lodz group¹⁸ indicates an excess of 1–2% from the galactic plane at $\sim 2 \times 10^{16} \text{ eV}$, a result which is consistent with the presence of 10–15 Cyg X-3-like sources in the Galaxy at the present epoch¹⁷.

Finally, we consider the latitude dependence of the X-ray flux due to the cosmic-ray electrons. The short lifetime of these electrons ($\sim 2 \times 10^3 \text{ yr}$) means that they will rarely travel beyond 100 pc from their production points. Now the z distribution of the sources is probably of a similar magnitude (young stars have a scale-height $z_1 \sim 70 \text{ pc}$; ref. 19), so that the z distribution of the energetic electrons is unlikely to be wider than that derived for the X-ray emissivity by Warwick *et al.*¹ from the experimental data.

All we now need is a test for the present hypothesis. The electrons, produced by the decay of charged pions, will have a differential exponent γ_d of 1.8 (for Cyg X-3 γ rays) to 2.8 depending on whether the target material, where the pions are produced, is thin or moderately thick. This exponent is likely to hold for electron energies of up to a few times 10^{15} eV , after which it may steepen sharply, as suggested by the spectral cutoff seen⁵ in the Cyg X-3 γ rays. As the synchrotron X rays ($< 10 \text{ keV}$) are produced by electrons of energy $10^{14}\text{--}10^{15} \text{ eV}$, we expect them to have a power-law spectrum with $\gamma_d = 1.4\text{--}1.9$. The observed spectrum may actually be steeper by $\Delta\gamma_d \approx 0.5$ because of the shorter lifetime of the higher energy electrons. The Exosat spectral data¹ seem to be consistent with thermal bremsstrahlung ($kT \sim 4\text{--}20 \text{ keV}$) or with a power law ($\gamma_d = 1.1\text{--}4.6$), and our prediction is in the middle of this range. It is to be hoped that better-quality spectral measurements will soon become available on the diffuse X-ray component and will further constrain the model spectrum.

The observation of the exponent as predicted here would go a long way towards establishing the phenomenon or, if the observed spectrum were steeper, in enabling an upper limit to be placed on the cosmic-ray electron contribution. In a sense, this test is complementary to the prediction by Rana *et al.*¹² of the presence of hard X rays, in the region of 100 keV, at high galactic latitudes. These X rays are likely to be due to synchrotron radiation by electrons, which are themselves produced in the halo following collisions of ultra-high-energy γ rays from Cyg X-3-like sources with the 3K radiation photons.

Received 2 August; accepted 30 September 1985.

1. Warwick, R. S., Turner, M. J. L., Watson, M. G. & Willingale, R. *Nature* **317**, 218–221 (1985).
2. Protheroe, R. J., Wolfendale, A. W. & Wdowczyk, J. *Mon. Not. R. astr. Soc.* **192**, 445–454 (1980).
3. Schwartz, D. A. in *COSPAR: X-Ray Astronomy* (eds Baity, W. A. & Peterson, L. E.) (Pergamon, Oxford, 1979).
4. Samorski, M. & Stamm, W. *Astrophys. J. Lett.* **268**, L17–L21 (1983).
5. Lloyd-Evans, J. *et al. Nature* **305**, 784–787 (1983).
6. Cooke, B. A., Griffiths, R. E. & Pounds, K. A. *Nature* **224**, 134–137 (1969).
7. Kearsley, S., Osborne, J. L. & Phillips, S. *IAU Symp.* **94**, 223–224 (1981).
8. Protheroe, R. J. & Wolfendale, A. W. *Astr. Astrophys.* **84**, 128–134 (1980).
9. Eichler, D. & Vestrand, W. T. *Nature* **307**, 613–614 (1984).
10. Hillas, A. M. *Nature*, **312**, 50–51 (1984).
11. Issa, M. R., Riley, P. A., Strong, A. W. & Wolfendale, A. W. *Astr. Astrophys.* **141**, 394–400 (1984).
12. Rana, N. C., Sadzinska, M., Wdowczyk, J. & Wolfendale, A. W. *Astr. Astrophys.* **141**, 394–401 (1984).
13. Bhat, C. L., Sapru, M. L. & Razdan, H. *Astrophys. J.* (submitted).
14. Protheroe, R. J. & Clay, R. W. *Nature* **315**, 205–207 (1985).
15. Wdowczyk, J. & Wolfendale, A. W. *Nature* **305**, 609–610 (1984).
16. Linsley, J. *Proc. 18th int. Conf. Cosmic Rays* **12**, 135–191 (1983).
17. Bhat, C. L., Kifune, T. & Wolfendale, A. W. *Astr. Astrophys.* (in the press).
18. Dzikowski, T., Gawin, J., Grochalska, B., Korejwo, J. & Wdowczyk, J. *Proc. 19th int. Conf. Cosmic Rays Vol. 1*, 238–241 (1985).
19. Allen, C. W. *Astrophysical Quantities* (Athlone, London, 1973).

An active current sheet in the solar wind

Steven J. Schwartz*, Chris P. Chalonert†, Peter J. Christiansen‡, Andrew J. Coates§, David S. Hall†, Alan D. Johnstone§, M. Paul Gough‡, Andrew J. Norris‡, Richard P. Rijnbeek||, David J. Southwood|| & Les J. C. Woolliscroft¶

* Theoretical Astronomy Unit, School of Mathematical Sciences, Queen Mary College, Mile End Road, London E1 4NS, UK

† Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX, UK

‡ School of Mathematical and Physical Sciences, University of Sussex, Brighton BN1 9QH, UK

§ Mullard Space Science Laboratory, Holmbury St Mary, Dorking, Surrey RH5 6NT, UK

|| Blackett Laboratory, Imperial College of Science and Technology, London SW7 2BZ, UK

¶ Physics Department, University of Sheffield, Sheffield S3 7RH, UK

The supersonic solar wind possesses a variety of simple small scale (<10-min observation duration) phenomena, including discontinuities^{1–3} and magnetic holes^{4,5}. We report here recent AMPTE-UKS⁶ (Active Magnetospheric Particle Tracer Explorer-UK Satellite) observations, however, which reveal a dramatic new complex structure within the solar wind flow. In the plasma and field observations of this magnetic hole/current sheet system, the layers surrounding the strong rotation in the magnetic field contain compressed and heated solar wind, an enhanced field strength and considerable wave activity. The interior region shows strong ion heating but with ambient densities and a fluctuating magnetic field which dips to low values. The bulk flow velocity inside is deflected sharply from the anti-sunward direction, but not in the direction predicted by magnetic stresses across the boundary. The structure is expanding in thickness and is relatively young. The source of momentum and energy for this event remains unclear, and could even be magnetospheric.

Most structures observed by *in situ* satellite measurements in the collisionless solar wind plasma have relatively simple, monotonic profiles and are either passive, convecting entities or shocks. The present observations seen by the AMPTE-UKS reveal an apparently more active current sheet. An important aspect of this work is the combination of high-time resolution three-dimensional particle data and wave data with the traditional magnetic field analyses on which earlier studies have been based. Recent, independent work⁷ using the ISEE spacecraft reports eight events which are very similar to the one presented here and provides a complementary range of data analysis.

Figure 1 displays five minutes of field and bulk plasma data taken in the solar wind on 30 October 1984. The spacecraft was

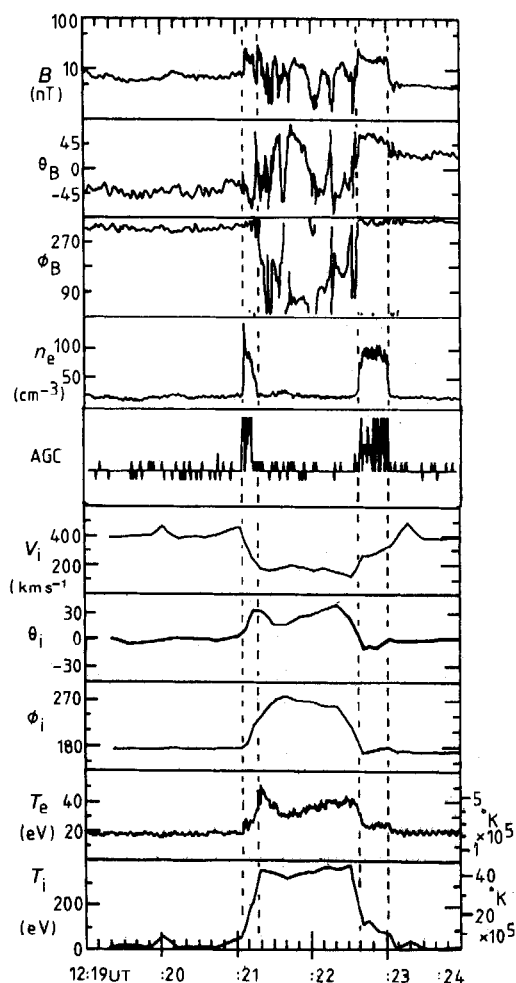


Fig. 1 Solar wind observations taken on 30 October 1984 by the AMPTE-UKS. Panels give (top to bottom) the magnetic field strength, latitude and azimuthal direction in geocentric solar ecliptic (GSE) coordinates (the sunward direction is $\theta = 0$, $\phi = 0$ with $\theta = 90$ being northward and $\phi = 270$ dawnward), electron number density, wave autocorrelator automatic gain control (AGC) level, ion flow velocity, flow latitudinal and azimuthal directions, and electron and ion temperatures. The electron measurements are based on individual spectra and could be averaged over one spin period (~ 5 s) to obtain the mean values. The ion parameters are taken on alternate spins when the ion instrument resolved either the solar wind component or uniform 4π coverage. The ion density, not shown, mimics n_e . The wave AGC level responds in successive factors of 2 of wave electric field amplitude in the 0.11–3.9 kHz band.

located in the dawn sector (approximately 09:30 LT) at a position relative to the Earth's centre of 11.3 Earth radii (R_E) sunward, 9.7 R_E dawnward and 0.22 R_E northward out of the solar ecliptic plane, placing it some 1.2 R_E upstream of the point where the satellite crossed the Earth's bow shock 80 min earlier. The magnetic field data are 0.5-s averages. The electron data are based on individual spectra covering the range 12 eV–16 keV taken at ≈ 1 -s intervals, with moments computed on the assumption of an isotropic distribution. Departures from isotropy thus appear as 5-s spin-modulated moments. The ion data shown have a time-resolution of ≈ 10 s.

On a longer timescale, the solar wind preceding the event, and until the penultimate dashed vertical line in Fig. 1, was disturbed by the presence of backstreaming ion beams and/or diffuse ions, indicating a magnetic connection to the Earth's bow shock. The magnetic field showed large amplitude fluctuations, with $\delta|B| \approx 2$ nT, about a mean field of $\langle |B| \rangle \approx 7$ nT. The large southward to northward rotation in latitude of the

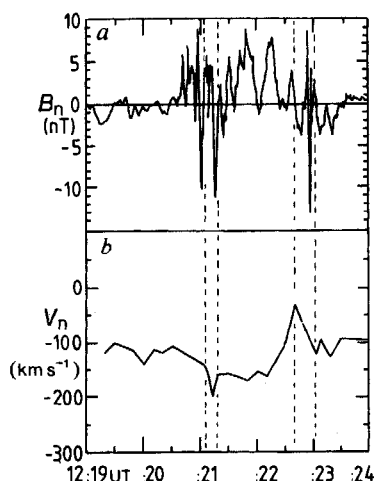


Fig. 2 Minimum variance component of the magnetic field (a) and corresponding ion velocity component (b). The normal direction is $\hat{n} \approx (0.38, 0.90, -0.22)$ in GSE (x, y, z) coordinates.

solar wind magnetic field from before to after the delineated event, seen in the second panel of Fig. 1, resulted in an exit from the ion foreshock, and a period of smooth solar wind with $\langle |B| \rangle \approx 5$ nT followed the interval shown here. The dashed vertical lines separate the obvious feature into three distinct regions.

The 'central' region is characterized by a varying magnetic field with several holes or depressions in field strength down to ≈ 1 nT. This is accompanied by hot electrons and even hotter ions, the latter dominating the total pressure here (see Table 1). The bulk flow shows a dramatic deceleration (from 400 to 150 km s⁻¹) and deflection (through $\approx 90^\circ$) from the ambient solar wind, resulting in a marked drop in bulk flow energy in the spacecraft frame of reference (see Table 1). Curiously the number density here returns to values comparable to those in the solar wind. The central region maintains rough pressure balance with the surrounding layers and is separated from them by a passive tangential discontinuity (the low central density eliminates the possibility of a shock transition).

'Edge' regions of enhanced field strength and density, accompanied by a partial deceleration, deflection and heating of the solar wind plasma but with little change in field direction,

Table 1 Pressures and energy densities (10^{-11} J m ⁻³)				
Parameter	Expression	Solar wind	Edge	Central
Magnetic pressure	$ B ^2/2\mu_0$	1.1	11	0.04–7.8
Ion thermal pressure	$n_i k T_i$	0.75	49	65
Electron thermal pressure	$n_e k T_e$	5.2	35	12
Normal bulk KE density	$\frac{1}{2} n_i 1.16 m_p V_n^2$	7.2	7.3	29
Transverse bulk KE density	$\frac{1}{2} n_i 1.16 m_p V - V_n \hat{n} ^2$	100	220	5.9

The ion densities used to compute this table were those actually measured and were a factor of 2–3 smaller than n_e due to differing experimental response/calibration.

* Assuming 4% helium by number.

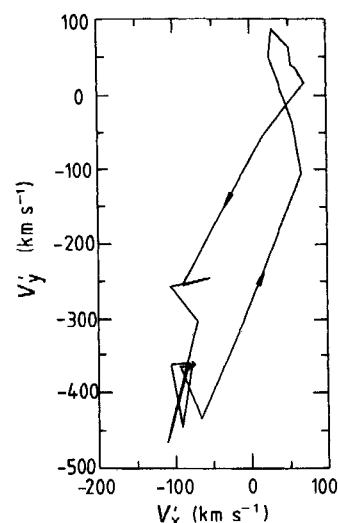


Fig. 3 Hodogram of the ion bulk velocity in the minimum variance plane of the magnetic field. The $\hat{x}'$ direction corresponds to the maximum variance eigenvector, $\approx (0.21, 0.14, 0.97)$ GSE, while $\hat{y}'$ is the intermediate eigenvector $\approx (0.90, -0.41, -0.14)$ GSE. Note that the velocity deflection is not aligned with the magnetic field maximum variance direction.

surround the central region. The automatic gain control (AGC) level of the wave experiment in this region gives r.m.s. wave amplitudes of the order of 1 mV m⁻¹ across this low-frequency band. Such strong fields usually indicate the presence of significant wave-particle interactions. Fast shocks probably form the outer boundaries of these regions although it is difficult to determine the downstream flow well enough to test this with any confidence. The particle correlator experiment on the satellite had sufficient time during the later trailing edge to reveal modulations over a range of frequencies (0–3 kHz) in the count rate of ≈ 100 eV electrons. This similarity to the response seen at the Earth's (fast) bow shock supports our supposition of a shock at the outer boundary. Table 1 shows that the total (plasma plus field) pressure in these edge regions is comparable to that of the central section, and that both considerably exceed the exterior pressure.

A minimum variance analysis of the field yields a well-defined normal direction (intermediate/minimum eigenvalue ≈ 4), $\hat{n}$, although Fig. 2 reveals quite strong fluctuations in the normal component of B . Figure 2 also shows the component of the ion bulk velocity along this normal. The average value of ~ 125 km s⁻¹ yields an estimated thickness of 15,000 km for the entire 2-min event. There is also a suggestion that the magnitude of V_n increases on entrance and decreases on exit, implying an expansion of the region by ± 50 –75 km s⁻¹. This interpretation is dependent on the normal direction which, given B_n from Fig. 2, may be inaccurate. Nonetheless, combining the expansion speed and thickness yields a maximum age of ~ 2 min for this active phase of the current sheet.

Figure 3 completes our analysis by displaying the ion bulk velocity in the plane of the current sheet. The large deflection of ~ 500 km s⁻¹ occurs primarily along the ($\hat{y}'$) intermediate variance direction. Tangential stress balance, however, (if $B_n \neq 0$) predicts that ΔV should be along the maximum variance direction $\hat{x}'$ (parallel to $\pm \Delta B$). Indeed, Fig. 2 has already indicated an average B_n which is consistent with zero. This central flow cannot correspond to the simple magnetic reconnection picture⁸ nor to that expected in smaller scale flux transfer events⁹ and must be bounded by purely tangential discontinuities. We conclude that the flow is driven predominantly by a strong pressure gradient within the sheet itself. Figure 4 summarizes the various field and flow directions deduced above.

The momentum and energy source responsible for the remarkable deflection and heating of the solar wind seen in this event

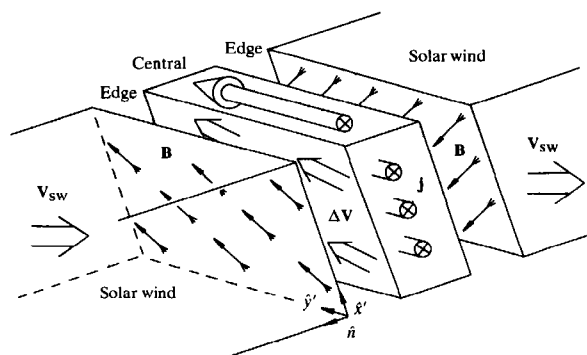


Fig. 4 Sketch of the geometry inferred from the data for the current sheet system, including the $(\hat{x}', \hat{y}', \hat{z})$ variance coordinate system of the magnetic field, $\mathbf{B}$ (thin arrows), along with the current density, $\mathbf{j}$ (cylindrical arrows), and bulk flow direction of the solar wind, $\mathbf{V}_{sw}$, and its deflection, ΔV , within the sheet (double arrows).

is not clear. This pressure pulse could have been generated by a fortuitously recent burst of magnetic reconnection located within the current sheet but some distance away from the satellite. On the other hand, we cannot eliminate the possibility of magnetospheric influence, most probably at the bow shock or within the magnetosheath. Tracing backwards along the velocity vector in the central region we reach a point of intersection with the bow shock (based on a fit to the bow shock¹⁰ normalized to its crossing 80 min earlier the same day). The time required to traverse this distance ($\sim 3.75 R_E$) is longer than our estimated maximal age, but only by 7%, which is well within our uncertainties in age, direction and bow shock position. Thomsen *et al.*⁷ discuss thoroughly (and cast doubt on) a variety of possible source hypotheses for their observed events.

Our data show a solar wind current sheet containing a strong, young pressure pulse which forces it to expand and drives a flow within the sheet itself. The hot, deflected interior is dominated by ion thermal pressure, and is separated from the ambient solar wind by regions of shocked or compressed solar wind. We have no explanation of the near solar wind densities inside the sheet, although initial compression/heating followed by the sheet's expansion may have conspired to provide this as a coincidence. We have not succeeded in identifying the energy source or triggering mechanism, which could be either magnetospheric or interplanetary in origin.

We have seen other examples in the AMPTE-UKS data set which display similar features to those presented here. A previously reported solar wind event¹¹ also showed some of the same characteristics.

We thank V. Formisano and our AMPTE-IRM colleagues from FRG for useful discussions, also Michelle Thomsen for comments and pre-publication material. We are indebted to Trefor Edwards, whose suspicions were aroused while watching the real-time AMPTE-UK data displays on 30 October 1984.

Received 15 July; accepted 6 September 1985.

- Burlaga, L. F. *Sol. Phys.* **4**, 67–92 (1968).
- Behannon, K. W., Neubauer, F. M. & Barnstorf, H. *J. geophys. Res.* **86**, 3273–3287 (1981).
- Russell, C. T., Gosling, J. T., Zwickl, R. D. & Smith, E. J. *J. geophys. Res.* **88**, 9941–9947 (1983).
- Turner, J. M., Burlaga, L. F., Ness, N. F. & Lemaire, J. F. *J. geophys. Res.* **82**, 1921–1924 (1977).
- Fitzzenreiter, R. J. & Burlaga, L. F. *J. geophys. Res.* **83**, 5579–5585 (1978).
- IEEE Trans. Geosci. Remote Sensing* **GE-23**, No. 3 (1985).
- Thomsen, M. F., Gosling, J. T., Fuselier, S. A., Bame, S. J. & Russell, C. T. *J. geophys. Res.* (submitted).
- Sonnerup, B. U. Ö. *Solar System Plasma Physics* Vol. III, 1.2, 45–108 (North-Holland, Amsterdam, 1979).
- Paschmann, G. *et al.* *J. geophys. Res.* **87**, 2159–2168 (1982).
- Slavin, J. A. & Holzer, R. E. *J. geophys. Res.* **86**, 11401–11418 (1981).
- Formisano, V. & Amata, E. *The Magnetospheres of the Earth and Jupiter* 205–217 (Reidel, Dordrecht, 1975).

A gravitational analogue of Faraday rotation

Tsvi Piran*† & Pedro N. Sañer*

*Racah Institute of Physics, The Hebrew University, Jerusalem, Israel

†Institute for Advanced Study, Princeton, New Jersey 08540, USA

In an effort to shed some light on the problem of nonlinear interaction of gravitational radiation we have studied the behaviour of cylindrical gravitational waves in vacuum. When only one mode of cylindrical gravitational waves is present, the well known solution is the Einstein–Rosen¹ waves which do not display any nonlinear behaviour. The general solution of two modes is unknown. To study it we express the cylindrical Einstein equations in a characteristic form and obtain an analytical approximation and a numerical solution for the general evolution of cylindrical waves. We report here that the propagation of these waves displays a reflection of ingoing to outgoing waves (and vice versa), combined with a rotation of the polarization vector between the + and × modes. The latter effect is a gravitational analogue of the electromagnetic Faraday rotation.

The general cylindrical Kompaneets–Jordan–Ehlers line element^{2,3}

$$ds^2 = e^{2(\gamma - \psi)}(dt^2 - dr^2) - e^{2\psi}(dz + \omega d\theta)^2 - r^2 e^{-2\psi} d\theta^2 \quad (1)$$

involves three functions ψ , ω , and γ , of r and t only. ψ and ω represent the two dynamical degrees of freedom of the gravitational field, + and × respectively; while γ plays the role of the total gravitational energy⁴. The Einstein equations in vacuum

$$\psi_{,tt} - \frac{\psi_{,r}}{r} - \psi_{,rr} = (e^{4\psi}/2r^2)(\omega_{,t}^2 - \omega_{,r}^2) \quad (2)$$

$$\omega_{,tt} + \frac{\omega_{,r}}{r} - \omega_{,rr} = 4(\omega_{,r}\psi_r - \omega_{,t}\psi_t) \quad (3)$$

$$\gamma_{,r} = r(\psi_{,t}^2 + \psi_{,r}^2) + \left(\frac{e^{4\psi}}{4r}\right)(\omega_{,t}^2 + \omega_{,r}^2) \quad (4)$$

$$\gamma_{,t} = 2r\psi_r\psi_t + \left(\frac{e^{4\psi}}{2r}\right)\omega_{,t}\omega_r \quad (5)$$

can be cast into a simpler characteristic form. To do so we define the ingoing and outgoing coordinates $u = \frac{1}{2}(t - r)$, $v = \frac{1}{2}(t + r)$; and the ingoing, I_+ , $I_\times$ and outgoing, O_+ , $O_\times$ amplitudes

$$I_+ = 2(\psi_t + \psi_r) \quad (6)$$

$$O_+ = 2(\psi_t - \psi_r) \quad (7)$$

$$I_\times = \frac{e^{2\psi}}{r}(\omega_t + \omega_r) \quad (8)$$

$$O_\times = \frac{e^{2\psi}}{r}(\omega_t - \omega_r) \quad (9)$$

The indices + and × denote the respective polarizations. With these definitions equations (2)–(3) become a set of four, first-order, coupled equations (R. F. Stark, personal communication):

$$I_{+,u} = \frac{I_+ - O_+}{2r} + I_\times O_\times \quad (10)$$

$$O_{+,v} = \frac{I_+ - O_+}{2r} + I_\times O_\times \quad (11)$$

$$I_{\times,u} = \frac{I_\times + O_\times}{2r} - I_+ O_\times \quad (12)$$

$$O_{\times,v} = -\left(\frac{I_\times + O_\times}{2r}\right) - O_+ I_\times \quad (13)$$

These equations are supplemented with the boundary conditions

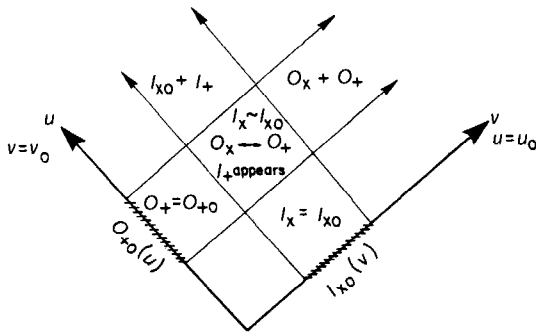


Fig. 1 Evolution for large r . The initial data are O_{+0} and I_{x0} . The initial waves propagate freely, until they cross each other. In the interaction region, O_+ and O_x rotate and a small amount of I_{x0} is converted to I_+ (see equations (17)–(20)). When leaving the interaction region, the rotation stops, and the ingoing and outgoing waves include both polarizations.

at the origin

$$I_+ = O_+ \quad \text{and} \quad I_x = -O_x \quad \text{at } r=0 \quad (14)$$

The initial conditions for equations (10)–(13) can be given either on a space-like hypersurface or on two null hypersurfaces, the latter being the simplest.

The first term on the right-hand side of equations (10)–(13), couples the ingoing and outgoing waves with the same polarization, giving rise to the usual cylindrical reflection. At $r=0$, this reflection is complete, and the ingoing waves turn into outgoing ones.

The other term describes a nonlinear interaction between the two polarizations. To investigate the nature of this interaction, we consider a large enough r , so that we can neglect the first terms in the right-hand side of equations (10)–(13). In this approximation, the solution of equations (10)–(13) becomes

$$I_x(u, v) + iI_+(u, v) = (I_{x0}(v) + iI_{+0}(v)) \exp \left[i \int_{u_0}^u O_x(u', v) du' \right] \quad (15)$$

$$O_x(u, v) + iO_+(u, v) = (O_{x0}(u) + iO_{+0}(u)) \exp \left[i \int_{v_0}^v I_x(u, v') dv' \right] \quad (16)$$

$I_{+0}(v)$, $I_{x0}(v)$, $O_{+0}(u)$ and $O_{x0}(u)$ are the initial conditions given on an outgoing ($u = u_0$) and an ingoing ($v = v_0$) null hypersurface respectively. When I_x is present, O_+ and O_x will oscillate according to equation (16) with a phase difference of $\pi/2$. This means that if the initial outgoing wave is linearly polarized its polarization vector will rotate as it propagates through the $I_x \neq 0$ region. The same is also true for the incoming waves (equation (15)).

For example, consider the case where $O_{x0} = I_{+0} = 0$ and $|O_{+0}| \ll |I_{x0}|$. I_x almost does not change:

$$I_x(u, v) \approx I_{x0}(v) \quad (17)$$

O_+ and O_x oscillate when the O_+ wave crosses the $I_x \neq 0$ region

$$O_+(u, v) \approx O_{+0} \cos \left(\int_{v_0}^v I_{x0}(v') dv' \right) \quad (18)$$

and

$$O_x(u, v) \approx -O_{+0} \sin \left(\int_{v_0}^v I_{x0}(v') dv' \right) \quad (19)$$

The appearance of O_x causes a conversion of a small part of I_x to I_+ and if $|O_{+0}| \ll 1$ we have

$$I_+(u, v) \approx -I_{x0} \int_{u_0}^u O_{+0} du' \sin \int_{v_0}^v I_{x0}(v') dv' \quad (20)$$

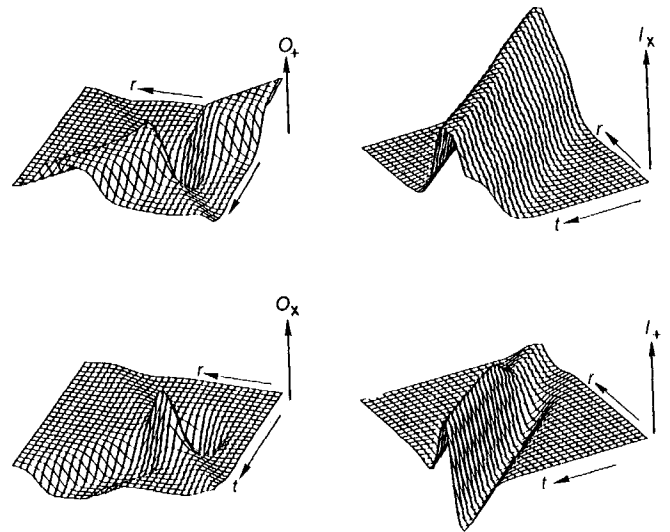


Fig. 2 I_x , I_+ , O_x and O_+ obtained by numerical solution⁶ of the exact equations (10)–(13). The initial data are $I_{+0} = O_{x0} = 0$, $|I_{x0}| \gg |O_{+0}|$, $|O_{+0}| \ll 1$. Note that the vertical scale is not the same in the different pictures. The behaviour matches the approximated equations (17)–(20) very well.

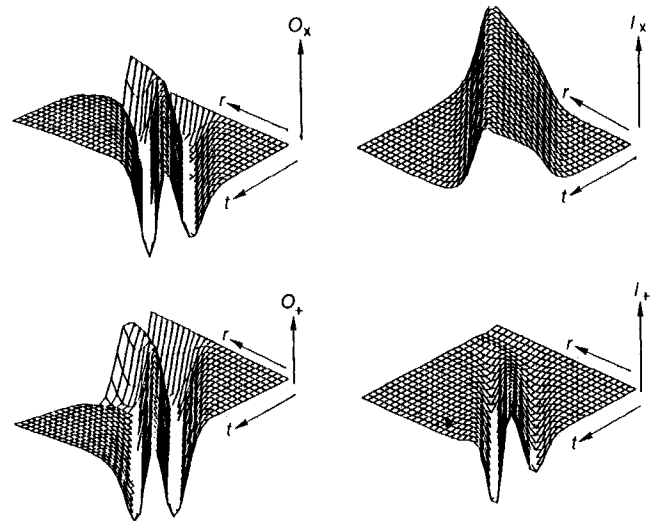


Fig. 3 Numerical results for initial data containing only an ingoing, $\times$ polarized pulse. Radiation in the I_+ , O_x and O_+ waves appears, and these waves rotate. Note that O_x and O_+ are out of phase by $\pi/2$. After O_x and O_+ leave the region where I_x is present the rotation stops (the vertical scaling is different for each section).

This behaviour is displayed in Figs 1 and 2. After the waves cross each other the rotation stops and the polarization vector freezes.

In general, when both O_{x0} and I_{x0} are large, each wave will cause a rotation of the polarization vector of the other.

The rotation of the polarization vector is due solely to the nonlinear nature of the gravitational interaction, that is, to the terms on the right-hand side of equations (2), (3). In electromagnetism there is no such interaction. However, in the presence of a magnetic field and plasma, the polarization vector of an incident linearly polarized wave rotates—a phenomenon known as Faraday rotation. Here the ingoing waves (say), play the role of both the plasma and the magnetic field as they rotate the polarization vector of the outgoing waves propagating through them.

One has to worry now about the combined effect of all terms in equations (10)–(13). If the initial data contains only the $+$ mode the sole effect will be the cylindrical reflection of I_+ to O_+ . These are the Einstein–Rosen waves. More complicated

behaviour emerges when the $\times$ mode is present in the initial data. We observe both conversion of I_+ to O_+ and $I_\times$ to $O_\times$ (via the first term in equations (10)–(13)) and rotation between I_+ and $I_\times$ and O_+ and $O_\times$ taking place simultaneously.

For example, an initial $I_\times$ pulse will reflect some $O_\times$ wave. This $O_\times$ wave will, in turn, cause a rotation of some of the initial $I_\times$ pulse into an I_+ pulse and by itself will rotate (due to the presence of $I_\times$) into O_+ (see Fig. 3).

Does this simple analysis overlook some features of the interaction of cylindrical gravitation waves, such as formation of singularities? To answer this question, with R. F. Stark, we have solved numerically equations (10)–(13). The exact solution (Figs 2, 3) demonstrate the validity of this simple picture and its completeness. A detailed description of these results will be given elsewhere⁵.

We thank S. Phinney and J. Katz for helpful discussions. This work was supported by NSF grant Phy. 84-07219 and by the BSF foundation.

Received 24 June; accepted 4 September 1985.

1. Einstein, A. & Rosen, N. *J. Franklin Inst.* 223, 43–54 (1937).
2. Jordan, P., Ehlers, J. & Kundt, W. *Acad. Wiss. Mainz. Abh. Math. Nat. Kl. Jahrg.* No. 2 (1960).
3. Kompaneets, A. S. *Soviet Phys. J. exp. theor. Phys.* 7, 659–660 (1958).
4. Thorne, K. S. *Phys. Rev.* 138, B251–256 (1965).
5. Piran, T., Safer, P. N. & Stark, R. F. *Phys. Rev. D* 15 (in the press).

Helium isotopes in North Sea gas fields and the Rhine rift

P. J. Hooker*, R. K. O'Nions & E. R. Oxburgh

Department of Earth Sciences, Downing Street,
University of Cambridge, Cambridge CB2 3EQ, UK

The popular assumption that all natural hydrocarbon accumulations are biogenic in origin has recently been questioned^{1,2} with the suggestion that deep source (mantle-derived) hydrocarbons may contribute to accumulated biogenically produced hydrocarbons, and also give rise to hydrocarbon deposits in unexpected parts of the crust. The possibility that deep-source volatiles are present in significant quantities in the hydrocarbon-bearing parts of the crust of western Europe is assessed here using analyses of ³He abundances in groundwaters and natural gas accumulations within the UK mainland and continental shelf. For comparative purposes, analyses are also presented for groundwaters from the southern part of the Rhine Graben, a region that has been relatively active tectonically, and the site of Tertiary and Quaternary volcanic activity. In contrast, the North Sea sedimentary basins and Central Graben have no recorded Tertiary or younger volcanic activity. Helium associated with North Sea gas fields and groundwaters on the UK mainland is dominantly radiogenic, produced by decay of U and Th in the continental crust, even where heat flow exceeds 100 mW m⁻². A minor component (<1%) of mantle-derived helium with primordial ³He, may be present in some gas fields close to the North Sea Central Graben. Further south along the more active part of the same fault system in the Rhine Graben this component reaches >15% in groundwater samples. Fault structures seem to be important for the transport of mantle helium through the brittle upper crust. The results are entirely consistent with a biogenic origin for North Sea hydrocarbons.

Table 1 and Fig. 1 show ³He/⁴He and He/Ne ratios for 10 natural gas samples from the UK shelf and mainland; analyses were made as described elsewhere³. Nine of the samples are from the North Sea basins including the Ninian, Frigg, Forties fields in the north, and Placid, Indefatigable, Viking, Dotty, Hewett and Leman in the south, and one sample is from the Wytch Farm field in southern England. He/Ne ratios are high,

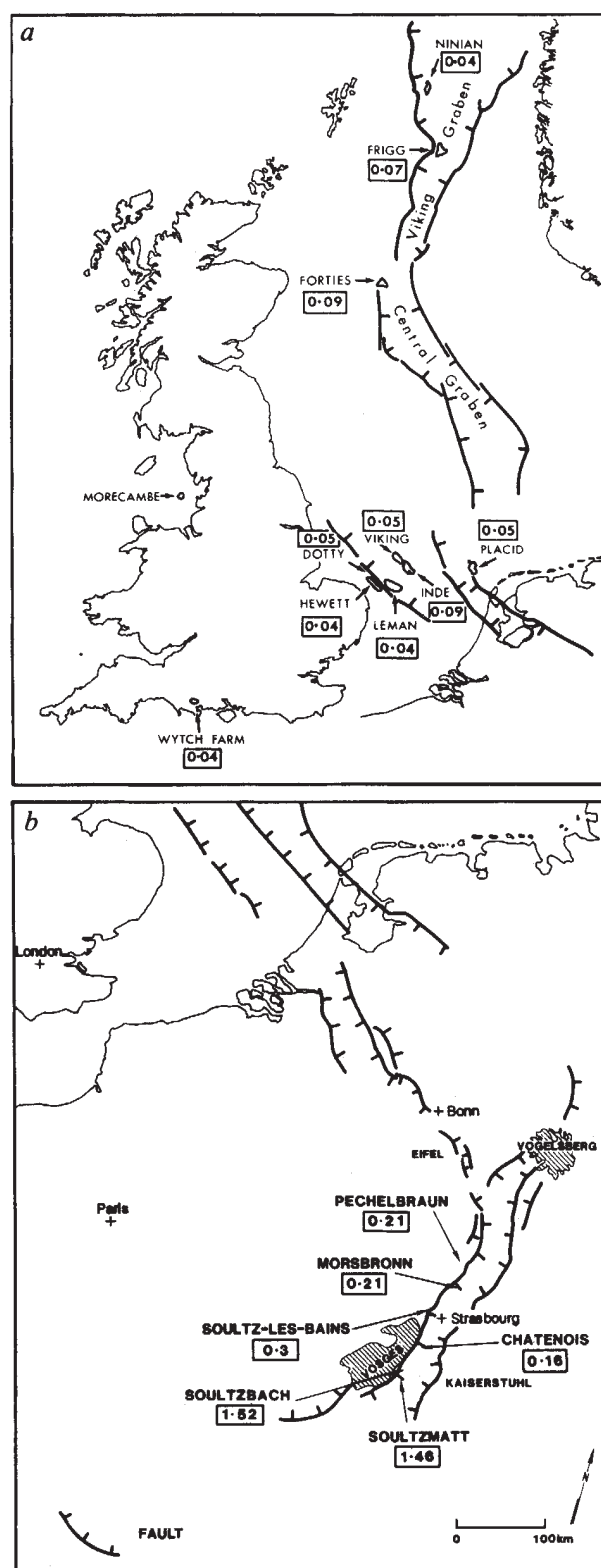


Fig. 1 a, Location of gas fields sampled for helium isotope analyses. b, Location of groundwater samples from the southern Rhine Graben. Numbers shown on maps are R/R_a values for samples analysed.

mostly >100, and indicate that any atmosphere-derived rare gas component is negligible in all cases except the Wytch Farm sample. Corrected $(R/R_a)_c$ ratios from the natural gas samples have a restricted range between 0.036 and 0.091, and where duplicate analyses have been made (Viking, Dotty, Indefatigable, Frigg and Ninian) the ratios agree within several per cent. (R/R_a is the ratio of the sample ³He/⁴He to that of the

* Present address: Fluid Processes Research Group, British Geological Survey, Keyworth, Nottingham NG12 5GG, UK.

Table 1 Helium results for natural hydrocarbon gas samples from the North Sea area and for groundwater samples from the Alsace region

Sample	R/R_a	He/Ne	$(R/R_a)_c$	[He]	Sample	R/R_a	He/Ne	$(R/R_a)_c$	[He]
Hydrocarbons					Groundwaters				
(10 ⁻⁹ nl per g H ₂ O)					(10 ⁻⁹ nl per g H ₂ O)				
G1473	0.045	350	0.044		Soultzmatt	1.35	100	1.35	19.5
Hewett					No. 2				
G1473 repeat	0.046	182	0.045		Soultzmatt	1.47	264	1.47	12.1
G1973	0.054	75	0.051		No. 3				
Viking					Soultzmatt	1.46	111	1.46	23
G1973 repeat	0.054	139	0.052		No. 4				
G2226	0.042	175	0.040		Soultzbach	1.53	168	1.53	13
Leman					No. 1				
G2147	0.047	142	0.045		Soultzbach	1.51	75	1.51	8.8
Dotty					No. 2				
G2147 repeat	0.048	122	0.046		Morsbronn	0.216	359	0.216	0.12
G2640	0.049	113	0.047		Arbogast No. 1				
Placid					Morsbronn	0.212	512	0.211	382
G2665	0.095	59	0.091		Currasier				
Indefatigable					Pechelbraun	0.212	480	0.212	263
G2665	0.089	305	0.089		71 °C				
new aliquot					Chatenois	0.153	64	0.150	101
G2390	0.099	10	0.072		Chatenois	0.159	311	0.159	439
Frigg					No. 2				
G2390	0.069	421	0.068		Soultz les Bains	0.301	177	0.300	1,230
new aliquot					Dept 67, No. 1				
G2706	0.037	302	0.036		Soultz les Bains	0.296	372	0.296	297
Ninian Field					Dept 67, No. 2				
(associated gas)									
G2706 repeat	0.036	495	0.036						
G2186	0.092	59	0.087						
Forties Field									
(associated gas)									
G2359	0.172	2.1	0.036						
Wytch Farm									
(associated gas)									

R/R_a is the ratio of the sample $^3\text{He}/^4\text{He}$ to that of air (1.4×10^{-6}). The sample He/Ne ratios ($\pm 10\%$) were used to correct for air-derived helium in the sample by the expression $(R/R_a)_c = [(R/R_a)X - 1]/(X - 1)$ where X is $(\text{He/Ne})_{\text{sample}}/(\text{He/Ne})_{\text{air or water } 10^\circ\text{C}}$. For the gas samples $(\text{He/Ne})_{\text{air}} = 0.288$ was used, and for the groundwaters $(\text{He/Ne})_{\text{water}} = 0.230$. Neon is regarded as being purely of atmospheric origin. Generally, errors in R/R_a are $\pm 3\%$ at the 95% level of confidence, in agreement with the comparisons afforded by the duplicates carried out on several of the hydrocarbon samples. The [He] values for the groundwaters have uncertainties of $\pm 10\%$.

atmosphere.) $^3\text{He}/^4\text{He}$ ratios of crustal helium sampled by groundwaters are similarly low. Samples from the Lincolnshire limestone aquifer in eastern England, the Marchwood borehole, Hampshire, and return waters from the Carnmenellis granite, artificially fractured as part of a Hot Dry Rock Project, have R/R_a values between 0.014 and 0.04 (Table 1, Fig. 2). The highest R/R_a values currently known from the UK mainland and shelf are from gas fields intimately associated with boundary faults to the Central Graben structure in the North Sea.

$^3\text{He}/^4\text{He}$ and He/Ne ratios are presented in Table 1 for 12 groundwaters from Alsace, several of which are currently exploited as mineral water sources. All samples have high He/Ne ratios, mostly > 100 , and have insignificant atmosphere-derived helium components, with higher and more variable R/R_a values ($0.14 < R/R_a < 1.5$) than those found on UK mainland and shelf. All of the Alsace samples show higher levels of ^3He -enrichment than any of the UK samples.

The presence of a small amount of mantle-derived helium in the continents should be recognizable even when diluted by very much larger amounts of radiogenic helium (Fig. 2). However, the estimation of its abundance depends critically on the precise R/R_a values assigned to the mantle and crustal components. The composition of the present-day mantle-derived component, typified by the release at spreading ridge axes, is well known, and more uncertainty surrounds the appropriate values for the radiogenic production ratio of helium in the crust. Early estimates of the production ratio⁴, which assumed the ^6Li (n, α) ^3H reaction to be the dominant ^3He -producing reaction, suggested R/R_a values for crustal helium of 0.1. More recent estimates⁵ of neutron-producing reactions in crustal rocks suggest a production value of $R/R_a = 0.02$, for typical continental

crust. In several situations, good agreement has been reported between the measured R/R_a values of helium and the predicted values for particular lithologies⁵. The above deductions are certainly consistent with the results reported for the groundwaters and for at least some of the hydrocarbon reservoirs in the UK, which contain helium derived from large rock volumes. Some departure from these 'typical' values is to be expected on the scale of individual minerals or fluid inclusions where large variations in Li abundance and in effective neutron flux, will occur; thus R/R_a values > 0.1 have been reported for particularly Li-rich minerals. Such high production ratios will not, however, be characteristic of large segments of continental crust, and the high values reported from Alsace are interpreted in terms of an admixture of mantle helium with crustal radiogenic helium. A few of the North Sea gas fields, particularly Forties and Indefatigable, have R/R_a values significantly greater than that predicted for crustal radiogenic helium, implying either a small component of mantle-derived helium mixed with 'normal' crustal helium, or the presence of crustal helium with an abnormal R/R_a value in the natural gas reservoir. The latter possibility is difficult to assess, but would presumably require that locally Mesozoic sediments have abnormally high Li-contents and that these are degassing preferentially, or alternatively Li-rich mineral components lose their helium selectively. In the absence of appropriate chemical information on the sediments themselves, this possibility cannot be excluded, but the close proximity and control of North Sea gas fields by fault structures, coupled with the observed large excesses in ^3He associated with similar structures in the southern Rhine Graben, suggests that a trace ($\sim 1\%$) component of mantle helium has entered the Forties and Indefatigable fields.

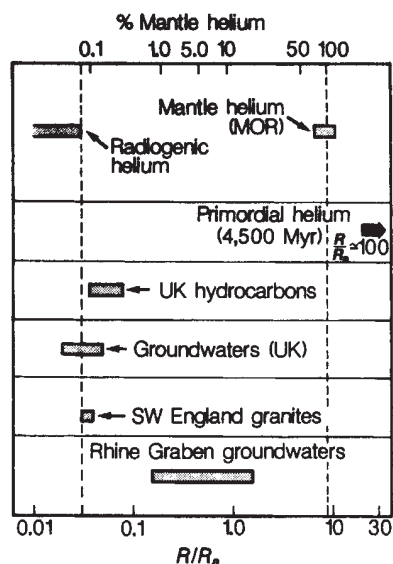


Fig. 2 The isotope composition of helium in samples from western Europe compared with radiogenic and mantle helium from mid-ocean ridges (MOR). $^3\text{He}/^4\text{He}$ ratios of samples (R) are compared with the $^3\text{He}/^4\text{He}$ ratio of the atmosphere, R_a ($=1.4 \times 10^{-6}$). The observed variations are interpreted as mixtures of radiogenic helium ($R/R_a < 0.03$) and mantle-derived helium possessing a primordial ^3He component with $R/R_a = 9$. The corresponding percentage of mantle helium on this basis is indicated at the top of the figure. The estimated composition of radiogenic helium is taken from ref. 5. Results for the Rhine Graben and UK hydrocarbons are from Table 1 and those for UK groundwaters are unpublished data from Cambridge and results of D. Hilton (personal communication). The south-west England granite data were obtained from a Hot Dry Rock experiment⁶.

The observed 50-fold variation in the R/R_a values of helium sampled from the UK and southern Rhine Graben raises the question of what factors control the transport of mantle-derived helium through the continental crust. The most obvious variables in the regional geology of the areas investigated are the heat flow, the presence of active faults associated with major rift structures, and the timing of the last volcanic episode within the area.

All helium results reported here from the mainland UK and others from south-west England⁶ and Lincolnshire (D. Hilton, personal communication) are from sites where the heat flow has been measured. Heat flow varies from $\sim 125 \text{ mW m}^{-2}$ in south-west England to $\sim 65 \text{ mW m}^{-2}$ in Lincolnshire⁷. R/R_a , however, remains close to 0.03 and shows no dependence on heat flow. This is the relationship to be expected in an old stable region where variation in heat flow depends largely on local variation in heat production in the crust. Although such variations in the abundance of U and Th do affect the heat flow they do not affect R/R_a , but only the total abundance of radiogenic helium. The heat flow in both the North Sea and the Rhine Graben is believed to be affected by deep fluid circulation⁸ and in the North Sea seems to vary with depth but in both areas may locally exceed 100 mW m^{-2} .

The igneous history of the three areas under discussion shows marked differences. Apart from Lundy in the English Channel, the southern part of the British Isles has not been affected by post-Mesozoic igneous activity. The same is probably true of the North Sea. The Rhine Graben, however, has a long history of activity⁹ and late Tertiary eruptions have occurred in the northern part of the Graben near the Eifel (11 Myr) and about 100 km south of Strasbourg at Kaiserstuhl (13 Myr). The highest R/R_a values came from localities close to the Kaiserstuhl. The whole of the Rhine Graben is seismically active today although the boundary faults close to which our samples were collected are relatively quiet; the greater part of the activity occurs away

from the margin fault, either just outside the Graben or clearly within it¹⁰. This difference has been tentatively attributed to the presence of fluids on the main boundary faults¹⁰. The Rhine Graben seismicity appears to weaken in intensity northwards and the Central Graben of the North Sea is nearly aseismic. The most intense seismic activity occurs in the Kaiserstuhl area where the highest R/R_a values are measured. All those samples that contain a component of mantle helium were collected near major faults. There is some suggestion that the highest mantle contributions occur where there is strongest activity. The very highest values come from an area of young igneous activity and high seismicity.

The importance of deep faults is that they are likely to provide transport paths for He through the brittle upper part of the crust which is too cool for diffusion to be effective. Elsewhere³ it has been suggested that the mantle-derived helium component associated with the high-temperature geothermal reservoirs at Larderello in northern Italy, has been advected to shallow (<6 km) levels in the crust by magma migration. In the Larderello region particularly, there is no recent volcanic activity at the surface, but a variety of lines of evidence indicate that a shallow ($\sim 7 \text{ km}$)-depth magma body is present and this is consistent with the large component of mantle helium observed near the surface. Similarly, in the southern Rhine Graben, it is possible that mantle helium is brought to comparatively shallow levels in association with magmas, and the faults act as channels for its escape to the surface. If it is real, the much smaller mantle helium component recognized in the North Sea may suggest either incompletely sealed deep fault or even possibly a signal of the onset of renewed activity.

The degradation of organic matter in sediments will be accompanied in space and time by the generation of radiogenic helium, which for typical sediments should have $R/R_a \approx 0.02$. Natural gases that have been sampled from the UK mainland and shelf have associated helium which is close to this value, consistent with a conventional source for the bulk of the hydrocarbons. Several of the North Sea gas samples have R/R_a values that are substantially higher than the expected radiogenic production ratio, which, if interpreted as an addition of a mantle-derived component, places an upper limit of 1% on the mantle helium component. In the case of the CH_4 -rich natural gases that border the Sea of Japan¹¹, the mantle-derived helium component may exceed 50%. Whether or not this might imply a volumetrically significant contribution of mantle CH_4 depends critically on the mantle $\text{CH}_4/^3\text{He}$ ratio and its modification during transport through the crust. On the basis of available observations on the volatile phase associated with oceanic basalts and mantle xenoliths^{12,13}, CH_4 must be considered as a minor species, far subordinate to CO_2 . Only if the dominant mantle-derived C species is CH_4 in continental regions, and $\text{CH}_4/^3\text{He} > 10^{10}$ is this source likely to be significant. Whereas there is the possibility of a trace of mantle-derived component in UK hydrocarbons, particularly close to the axial graben of the North Sea, there is no reason at present to conclude that the major gas CH_4 has anything but a conventional biogenic origin.

Different aspects of this research have been supported by the British Gas Corporation, EEC (contract no. EG-A-3-004-UK(N)) and the Royal Society. We thank Dr Meunier (University of Strasbourg) for assistance in sampling in Alsace. This is Department of Earth Science contribution no. 619.

Received 20 May; accepted 17 September 1985.

- Gold, T. A. *Rev. Energy* 10 (in the press).
- Gold, T. & Soter, S. *Scient. Am.* 242, 154–161 (1980).
- Hooker, P. J., Bertrami, R., Lombardi, S., O'Nions, R. K. & Oxburgh, E. R. *Geochim. cosmochim. Acta* (in the press).
- Morrison, P. & Pine, J. *Ann. N.Y. Acad. Sci.* 62, 69–92 (1955).
- Mamyrin, B. A. & Tolstikhin, I. N. *Helium Isotopes in Nature* (Elsevier, Amsterdam, 1984).
- Hilton, D., Oxburgh, E. R. & O'Nions, R. K. *Inst. Min. Metall.* (submitted).
- Oxburgh, E. R. & Richardson, S. W. *Nature* 282, 565–567 (1979).
- Andrews-Speed, C. P., Oxburgh, E. R. & Cooper, B. A. *Am. Ass. Petrol. Geol.* 68, 1764–1781 (1984).
- Neugebauer, H. J. & Temme, P. *Tectonophysics* 73, 33–51 (1981).
- Illies, J. H. *Tectonophysics* 73, 249–266 (1981).
- Wakita, H. & Sano, Y. *Nature* 305, 792–794 (1983).
- Roedder, E. *Am. Miner.* 50, 1746–1782 (1965).
- Andersen, T., O'Reilly, S. Y. & Griffin, W. L. *Contr. Miner. Petrol.* 881, 72–85 (1984).

Relics of H₂O fluid inclusions in mantle-derived olivine

Shoji Arai & Hisako Hirai

Institute of Geoscience, University of Tsukuba, Ibaraki 305, Japan

In olivine of upper mantle derivation, CO₂ is the most commonly reported species of fluid¹. Despite its great importance in the upper mantle², H₂O has rarely been reported (refs 3, 4 and E. Roedder, personal communication). Here we report that possible relics of H₂O-rich fluid inclusions have been found in olivine of dunite from the alpine-type Iwanai-dake complex, northern Japan. These inclusions now consist mainly of serpentine and brucite, which may be the products of the reaction, olivine + H₂O = serpentine + brucite, in a closed system. The abundance of such inclusions suggests that the Iwanai-dake complex may have been derived from the upper mantle in conditions in which some free H₂O was available, such as the mantle wedge above a subduction zone.

The Iwanai-dake peridotite complex was emplaced in the southern part of Kamuikotan zone, located at the central zone of Hokkaido. The Kamuikotan zone is characterized by the tectonic mixture of high-pressure (jadeite–glaucophane type) and ocean-floor type low-pressure metamorphic rocks⁵. An ophiolite complex with the basal harzburgite was found at Horokanai⁶, the northern part of the Kamuikotan zone, and was interpreted to be a slice of the Mesozoic oceanic lithosphere which had been consumed to give rise to the collision between the Eurasian plate and the North American plate (the Kurile arc) in the Miocene⁷. However, large quantities of ultramafic rocks, especially those at the southern part of the Kamuikotan zone including the Iwanai-dake complex, constitute, not ophiolitic sequences, but isolated peridotite complexes. The Iwanai-dake complex^{8,9} is composed mainly of roughly layered dunite and harzburgite of upper mantle origin, with very small amounts of chromitite and pyroxenites. Application of the olivine–spinel geothermometry and geospeedometry¹⁰ gives an initial equilibrium temperature of 810 °C (or a little higher) from which the complex had cooled by 200 °C at the rate of 10⁻²–10⁻⁴ °C yr⁻¹. The peridotites around Iwanai-dake are almost wholly devoid of serpentinization and the degree of serpentinization increases abruptly towards the margin of the complex, indicating the post-emplacment nature of the serpentinization. Dunite and harzburgite are strongly magnesian and contain olivines of Fo₉₅₋₉₁ and Fo₉₃₋₉₁, respectively, characterized by exsolution lamellae of chromian spinel⁸.

Olivine is also characterized by many tiny inclusions, which form arrays in thin section (Fig. 1). These inclusions were examined in a particular sample of dunite (IW-8407) composed of equant coarse (up to 1 cm diameter) olivine (Fo₉₄₋₉₃, Fo_{93,6} on average) and euhedral chromian spinel (Cr/Cr + Al atomic ratio, 0.75).

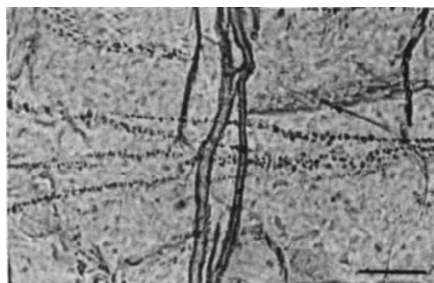


Fig. 1 Photomicrograph of inclusions in olivine, taken in plane-polarized light. Inclusion arrays are sharply cut by the post-emplacment serpentine veins. Scale bar, 50 μ m.

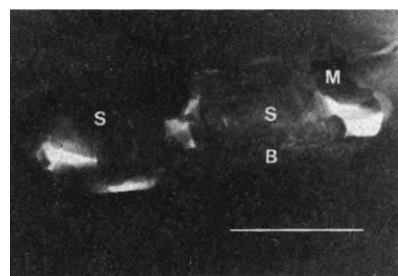


Fig. 2 Transmission electron micrograph of an inclusion containing serpentine, brucite and magnetite (indicated by S, B and M, respectively). Note that the inclusion is completely surrounded by host olivine. Scale bar, 1 μ m.

Some inclusions crosscut the kink band, suggesting that they formed after recrystallization of the host olivine. The shape of an individual inclusion is typically columnar. The inclusion arrays are clearly cut by post-emplacment serpentine (PES, chrysotile–lizardite), but do not cut or distort the fine texture of the serpentine vein. An individual inclusion can be shown to be isolated in the host olivine (Fig. 1).

The ion-thinned foils were observed and analysed in JEOL 200-CX electron microscope¹¹. The inclusion (Fig. 2) is completely surrounded by the host olivine and has a clear-cut outline of hexagonal shape, forming a sharply faceted negative crystal (Fig. 2) elongated parallel to [100] of the host olivine. Electron diffraction (Fig. 3a, b) and semi-quantitative analysis revealed that serpentine (S in Fig. 2), which forms bundle and radial fibrous aggregates, and brucite (B in Fig. 2), which is a rhomboid extended along the inclusion wall, are the main constituents of the inclusion. Notably, the volume ratio of serpentine to brucite is approximately 4 (Fig. 2). Species of the serpentine mineral could not be distinguished. A minute grain of Fe–Ni alloy or magnetite was always found (Fig. 2).

Because the present inclusion is completely surrounded by the host olivine, it is unlikely to have formed as a result of an external source of water at temperatures low enough for serpentine to remain stable. It is also improbable that the inclusion of hydrous minerals represents an early stage of the PES of the rock, because the inclusion arrays do not affect the fine texture of the serpentine vein (Fig. 1). We know of no plausible mechanism for the formation of such inclusions in olivine at the even lower temperature that would prevail after the PES. Nor is the array of inclusions a partially healed veinlet of serpentine and brucite, as no traces suggestive of the hydration–dehydration stage before the PES (for example, the paragenesis of olivine + magnetite or ferritchromite) could be found. The most appropriate interpretation is that, when the temperature (and possibly pressure) decreased, the water vapour trapped before the PES reacted with the surrounding olivine to form hydrous minerals via reactions such as $2\text{Mg}_2\text{SiO}_2$ (olivine) + $3\text{H}_2\text{O} =$

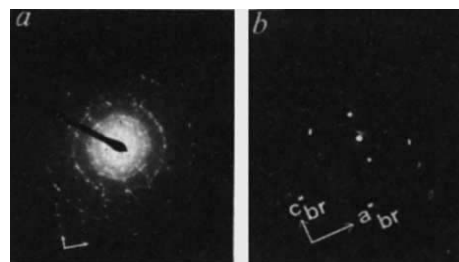


Fig. 3 Electron diffraction patterns of serpentine (a) and brucite (b). Debye rings and streaks observed in a can be indexed as reflections of serpentine. A net-like pattern in the lower left-hand of a (indicated by arrows) is that of $hk0$ of host olivine (shorter and longer arrows, b^* and a^* , respectively).

$\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_2$ (serpentine) + $\text{Mg}(\text{OH})_2$ (brucite)¹². This reaction gives a volume ratio of serpentine to brucite of ~4.4 (ref. 13), which is consistent with that observed (~4). Since magnesite was not found in the inclusions examined, the $\text{CO}_2/\text{H}_2\text{O}$ ratio of the fluid must have been very low.

The present relic H_2O -rich fluid inclusions show the characteristics of so-called secondary inclusions¹, which implies that they were formed by healing of the fracture in olivine which had been filled by water vapour. The inclusion was formed at temperatures >400 °C, below that at which the ordinary serpentinization had occurred¹², and after the main deformation stage, which occurred at high temperatures. It is probable that the Iwanai-dake complex was derived from the relatively low-temperature upper mantle where free H_2O was available. This situation could be most easily achieved at the mantle wedge^{14,15}, especially near the leading edge, above a downgoing slab, where free H_2O in addition to CO_2 may be generally available. For example, peridotite xenoliths from the Ichinomegata crater (400 km on the continent side of the Japan Trench)^{16,17}, northern Japan, derived from the hydrated mantle wedge beneath the North-East Honshu Arc¹⁸, are the only known mantle materials containing H_2O -bearing fluid inclusions^{3,4}. In contrast, other peridotite xenoliths containing almost pure CO_2 fluid inclusions were derived from the upper mantle free of underlying subduction zones⁴. Olivine in peridotites of the mantle wedge, if slowly cooled to low temperatures, would have inclusions of serpentine-brucite, serpentine-brucite-magnesite, serpentine-talc-magnesite and talc-magnesite-enstatite, depending on the $\text{H}_2\text{O}/\text{CO}_2$ ratio of the fluid¹⁹. Peridotites in which H_2O -rich fluid inclusions constitute the leading edge of the mantle wedge

(or the fore-arc mantle) may be emplaced as ophiolites or alpine-type masses²⁰ like the Iwanai-dake complex, during the arc-arc collision in Hokkaido in the Miocene¹. Peridotites of the typical fore-arc ophiolite, such as the Papuan Ultramafic Belt²¹, would be expected to contain olivine with serpentine-brucite inclusions.

We thank Drs E. Roedder, R. J. Arculus, T. Fujii and M. Kimata for their suggestions.

Received 30 July; accepted 13 September 1985.

1. Roedder, E. in *Fluid Inclusions*, 11–26, 503–532 (Mineralogical Society of America, Washington D.C., 1984).
2. Ito, E., Harris, D. M. & Anderson, A. T. Jr. *Geochim. cosmochim. Acta* 47, 1613–1634 (1983).
3. Roedder, E. *Am. Miner.* 50, 1746–1782 (1965).
4. Trial, A. E., Rudnick, R. L., Ashwal, L. D., Henry, D. J. & Bergman, S. C. *EOS* 65, 306 (1984).
5. Ishizuka, H., Imaizumi, M., Gouchi, N. & Banno, S. *J. metamorphic Geol.* 1, 263–275 (1983).
6. Ishizuka, H. *J. Geol. Soc. Japan* 86, 119–134 (1980).
7. Kimura, G., Miyashita, S. & Miyasaka, S. in *Accretion Tectonics in the Circum-Pacific Regions* (eds Hashimoto, M. & Uyeda, S.) 123–134 (TERRAPUB, Tokyo, 1983).
8. Arai, S. *Earth planet. Sci. Lett.* 39, 267–273 (1978).
9. Nagata, J. *J. Japan Ass. Miner. Petrol. econ. Geol.* 77, 23–31 (1982).
10. Ozawa, K. *Geochim. cosmochim. Acta* 48, 2597–2611 (1984).
11. Hirai, H. & Arai, S. *A. Rep. Inst. Geosci. Univ. Tsukuba* (in the press).
12. Evans, B. W. A. *Rep. Earth planet. Sci.* 5, 397–447 (1977).
13. Helgeson, H. C., Delaney, J. M., Nesbitt, H. W. & Bird, D. K. *Am. J. Sci.* 278-A, 1–229 (1978).
14. Tatsumi, Y., Sakuyama, M., Fukuyama, H. & Kushiro, I. *J. geophys. Res.* 88, 5815–5825 (1983).
15. Honda, S. *Tectonophysics* 112, 69–102 (1985).
16. Kuno, H. in *Ultramafic and Related Rocks* (ed. Wyllie, P. J.) 337–342 (Wiley, New York, 1967).
17. Aoki, K. *Contr. Miner. Petrol.* 30, 314–331 (1971).
18. Takahashi, E. *Bull. Volcanol.* 41, 529–547 (1978).
19. Johannes, W. *Am. J. Sci.* 267, 1083–1104 (1969).
20. Moores, E. M., Robinson, P. T., Malpas, J. & Xenophonotos, C. *Geology* 12, 500–503 (1984).
21. Davies, H. L. & Jaques, A. L. in *Ophiolites and Oceanic Lithosphere* (eds Gass, I. G., Lippard, S. J. & Shelton, A. W.) 341–349 (Geol. Soc./Blackwell, Oxford, 1984).

Improved kerogen typing for petroleum source rock analysis

Stephen R. Larter & Joseph T. Senftle

Unocal Science and Technology Division, Unocal Corporation, Exploration Research, 376 South Valencia Avenue, Brea, California 92621, USA

The chemical characterization of kerogen (typing) is a routine part of petroleum exploration programmes¹. Traditionally, kerogen has been characterized on the basis of microscopy² and bulk chemical methods³, procedures derived from the coal characterization literature^{1,4}. These methods do not today, however, provide adequate resolution of the complex mixture of kerogen components encountered in source rocks from petroleum-bearing sedimentary basins⁵. Here we report an improved classification approach for kerogens at all maturity levels. The method is based on a quantitative pyrolysis-gas chromatography approach using a polymeric internal standard, polymethylstyrene, to directly determine the absolute concentrations of specific kerogen pyrolysis products. In particular, cross-plots of normal hydrocarbon, aromatic hydrocarbon and total hydrocarbon yields of kerogen pyrolysis are used to recognize eight separate nodes of kerogen composition.

Two major factors control the petroleum potential of any sediment at any maturity level: (1) the quantitative fraction of the sediment that contains potential petroleum-related chemical structures, and (2) the efficiency of expulsion of these petroleum species from the source bed^{1,5}. The maturity level of organic matter in a sediment simplistically reflects its extent of progression along the reaction coordinate system representing reactions converting sedimentary organic matter (immature) to oil and gas (mature) and ultimately methane and graphite (post-mature).

The fraction of the sediment that contains catagenetically releasable petroleum-related structures is correlated closely with the kerogen type present in the sediment^{1,3,5}. Pyrolysis-gas

chromatography (pyrolysis-GC) is widely used both to elucidate the structure of kerogens^{5–9} and to type them according to chemical properties and physical characteristics^{5,6–14}, with a view, among other applications, to determining catagenic petroleum yield and composition¹. However, conventional pyrolysis-GC fingerprinting of kerogens is a rather subjective process and cannot easily resolve differences between the continuous series of mixed type II and type III kerogens often found in near-shore marine depositional settings. Ideally, kerogens should be characterized at the same molecular level, as is possible with rock extracts and crude oils; thus, full quantitation of all pyrolysis products (as may be possible with calibrated pyrolysis-mass spectrometric methods) is desirable. Here, we have used pyrolysis-GC to quantitate normal hydrocarbon yields from kerogens on pyrolysis, and these data are used to better define kerogen type and, hence, petroleum yield and type.

Normalized abundances of pyrogram (pyrolysis-gas chromatogram) components were obtained on a per-kerogen or per-carbon basis, using a polymethylstyrene internal standard that was added and mixed in dichloromethane solution with the extracted kerogen. Pyrolysis-mass spectrometry and thermogravimetric analysis showed that the standard polymer decomposed during pyrolysis, at temperatures well below the maximum temperature (800 °C), to yield a monomer (methylstyrene) as predominant product. The use of a polymeric internal standard is necessary due to the nature of the pyrolysis system used, in which sample loss during instrument set-up sometimes occurs and where a volatile standard would be lost non-quantitatively from the sample before pyrolysis. The device is described in Fig. 1 legend and is documented elsewhere¹². Polystyrene has been used in quantitative pyrolysis-GC studies of soils¹⁵ but the common presence of styrene (the pyrolysis product of polystyrene) as a kerogen pyrolysis product would interfere with the use of polystyrene here. Methylstyrene, while a minor component of some coal maceral kerogen pyrolysates^{12,13}, provides a component that is generally at a low concentration in most pyrolysates, and which elutes chromatographically away from other peaks of interest in pyrograms of most source rock kerogens.

Fig. 1 Normalized abundance (by weight of kerogen) of aliphatic and aromatic hydrocarbons in low-maturity kerogen pyrolysates. The diagram represents a cross-plot of the abundance of C_7 - C_{25} normal hydrocarbons (alkenes plus alkanes) and of C_6 - C_8 alkylaromatic hydrocarbons (benzene, toluene, ethylbenzene, xylenes, styrene) obtained by pyrolysis at 800 °C for 20 s of up to 500 µg of solvent-extracted kerogen. □, Type I: alginites from Australia. △, Type II: Miocene, California; Triassic, North Sea; Jurassic, Paris Basin; Jurassic, North Sea; Tertiary, Alaska; Carboniferous, UK. ○, Type III: Jurassic, North Sea; Miocene, Indonesia; Cretaceous, Canada. ×, Type IV: Carboniferous, UK.

Methods. The kerogens were pyrolysed on a platinum filament in flowing helium on a Chemical Data Systems 120 Pyroprobe, modified for use with capillary GC columns¹². Kerogen/poly-methylstyrene mixtures were placed on the cleaned pyrolysis filament in methanol suspension via a glass capillary tube; the methanol was evaporated before pyrolysis. The procedure used is described in ref. 12. GC analysis was performed via a splitter system, the pyrolysate being separated on a 50-m SP2100 capillary column programmed from 40 to 300 °C at 4 °C min⁻¹. Hydrocarbons were quantitated using a methylstyrene internal standard generated during the pyrolysis process from polymethylstyrene admixed with the kerogen. Automated peak area integration of the pyrograms was done on a microcomputer. Replicate analyses of aliquots of the samples allowed determination of the reproducibility of the method (error bars represent 2σ variation about the mean). The kerogens used were all of maturity less than that equivalent to a vitrinite reflectance level of 0.8%. One standard unit approximately equals 0.25%, by weight, of kerogen.

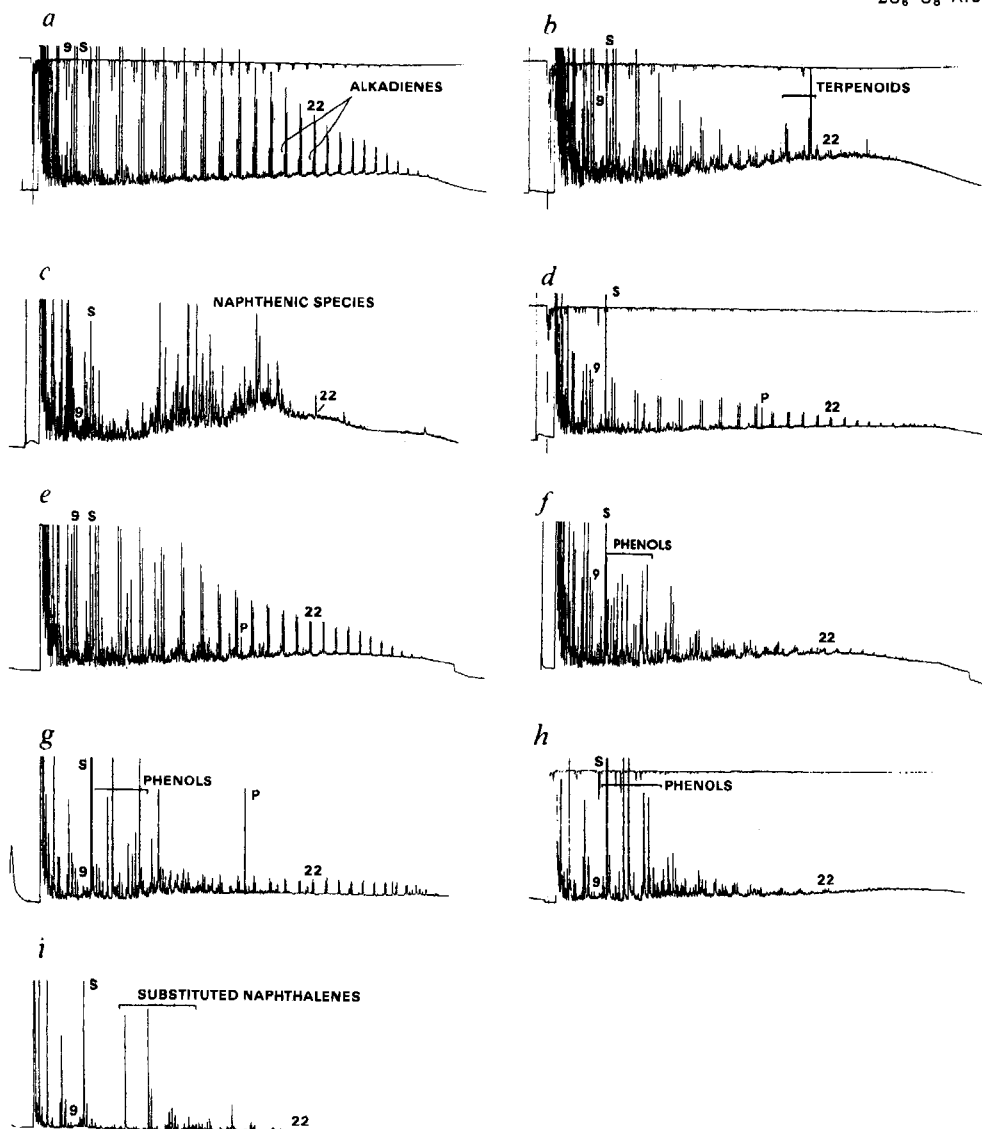
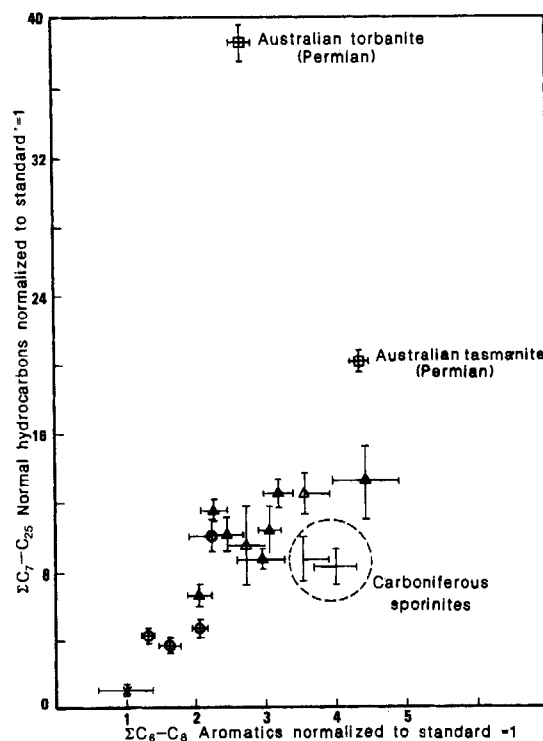


Fig. 2 Principal kerogen types as defined by pyrolysis-GC. The figure shows capillary pyrograms of nine kerogens, all with maturity levels <0.8%, vitrinite reflectance equivalent. The complete kerogen type description consists of the following: (1) A prefix denoting bulk chemical type, based on the work of Tissot *et al.*³; (2) a suffix describing the qualitative pyrogram appearance and quantitative paraffin abundance in the pyrogram; (3) a petrographic descriptor indicating the principal maceral component identified on reflected, transmitted and ultraviolet light examination of kerogen concentrates. A complete description of, for example, sample 1 (a) is, therefore, type I (Tissot type), p (highly paraffinic pyrolysate), alginite (principal kerogen component) = type Ip alginite (sample 1 in Fig. 3: Permian torbanite, Australia). b, Type Ipn alginite (sample 3 in Fig. 3: Permian tasmanite, Australia). c, Type In resinite (sample 4 in Fig. 3: Tertiary resinite, Indonesia). d, Type Iipn amorphinite¹⁷ (sample 7 in Fig. 3: Jurassic Kimmeridge Clay Formation, North Sea). e, Type II/IIIp vitrinite/exinite (sample 15 in Fig. 3: Permian, Kupferschiefer Formation, North Sea). f, Type II/IIIpp sporinite (sample 17 in Fig. 3: Carboniferous coal sporinite, Yorkshire, UK). g, Type IIpp vitrinite (sample 24 in Fig. 3: Tertiary, Indonesia). h, Type IIIap vitrinite (sample 28 in Fig. 3: Carboniferous coal vitrain, Pennsylvania). i, Type IV fusinite (sample 29 in Fig. 3: Carboniferous fusain, Yorkshire). The positions of the internal standard methylstyrene (S) and the C₉ (9) and C₂₂ (22) n-alkenes and alkanes and P, prist-1-ene are also indicated. Column conditions as for Fig. 1.

Figure 1 shows the absolute yield of aromatic (expressed as total benzene, toluene and C_8 aromatics) and normal hydrocarbons (C_7 – C_{25} n -alkenes plus n -alkanes) for a variety of low-maturity kerogens. Component abundances were determined by automated peak recognition and integration on a laboratory microcomputer system. Interestingly, the absolute normal hydrocarbon content of several of the type II and III kerogens is similar. In general, it is the higher naphthenic contribution from marine organic matter that provides the increased hydrogen indices of marine kerogen systems. Replicate analysis reveals that some approach to accurate quantitation of kerogen pyrolysis products can be made rapidly in this manner and that the method may prove applicable to more general quantitative characterization of complex geopolymers.

While fingerprint approaches have been successfully applied to kerogen typing using pyrograms^{6,8-10,12,13}, it is the lack of high-resolution quantitative data that has precluded typing kerogens to the same level of resolution with which source rock bitumens or oils are characterized¹. Figure 2 shows pyrograms of several kerogens which we believe represent distinct molecular assemblages at low maturity levels. Figure 3 shows a cross-plot of a hydrogen index parameter (determined from elemental analysis data; compare with ref. 16) and the total organic carbon (TOC)-normalized abundance of C_9 – C_{30} normal hydrocarbons estimated from peak height data. It is the relative positions of the kerogens on this diagram, the pyrogram fingerprint character, and a knowledge of the petrographic properties of the kerogens that allow this more refined subdivision of the classical Tissot typing scheme³. The inclusion of petrographic and chemical data in the characterization of source rock kerogens is widely practised within petroleum geochemical circles¹ and is a critical requirement when quantitative assessments of source rocks are required in terms of volumes of oil sourced from a source bed⁵.

By directly determining the total reservoir of specific pyrolysis elements present in the kerogen at any maturity level in the manner described above, direct assessment of actual petroleum potential for any kerogen system becomes possible on a molecular level. Further, this method allows the discrimination of kerogens hitherto grouped together on the basis of elemental analysis or hydrogen/oxygen indices. Thus, in Fig. 2g, h, the two type III kerogens analysed, while petrographically vitrinite-dominated, are clearly different in detailed composition. Vitrinites from the deltaic system (Fig. 2g) have appreciable quantities of normal hydrocarbon precursors in the system while the vitrinite from a coal swamp environment (Fig. 2h) is paraffin precursor-depleted. In assessing hydrocarbon-generating potential for a kerogen assemblage, it is frequently difficult to deduce actual potential from bulk chemical analyses as many detailed compositions, some capable of generating components of high molecular weight, are possible for any given bulk chemical analysis.

The detailed composition of kerogen pyrolysates as a function of type has been extensively reviewed before⁸⁻¹³ with regard to normal hydrocarbon and hydrogen index variation, as summarized below and in Fig. 3. Type I kerogens (Figs 2, 3) are generally characterized at immaturity with high hydrogen indices but the thermal degradation products may be highly paraffinic (Ip), mixed paraffinic/naphthenic (Ipn), or naphthenic (In), depending on source facies^{6,12} (examples are shown in Fig. 2). Type II kerogens with intermediate hydrogen indices and relatively low paraffin contents (IIpn) are characteristic of kerogens from marine source beds containing dominantly amorphous organic matter derived from algal and bacterial sources². Kerogens from many clastic 'super-source rocks' fall into this category, examples being kerogens from the Kimmeridge Clay Formation (North Sea basins), Lower Cretaceous sediments from the North Slope basins of Alaska, Miocene sediments from the Tertiary basins of southern California, and Lower Jurassic sediments from the Paris Basin. Type II kerogens with intermediate hydrogen indices but high paraffin precursor contents (IIp) are generally associated with land plant-derived exinite-rich systems.

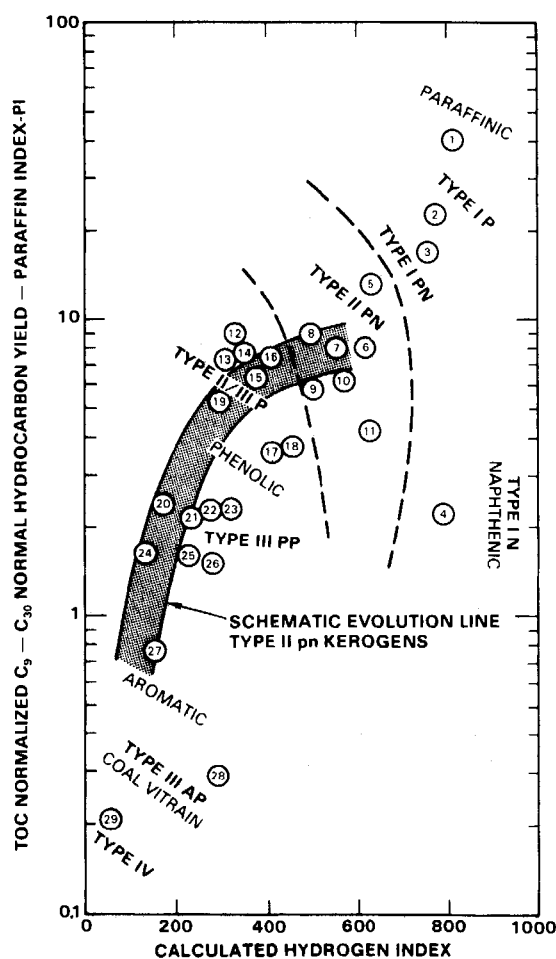


Fig. 3 Paraffin index/hydrogen index cross-plot. The figure shows a cross-plot of the summed abundance of the C_9 – C_{30} n -alkenes plus n -alkanes determined from pyrogram peak height data plotted against a computed hydrogen index derived from elemental analysis data in the manner of Orr¹⁶ (Rockeval units = mg hydrocarbons per g TOC). Although not a true hydrogen index determined analytically, this approach does allow sample comparison on a common matrix effect-free basis. Paraffin index is based on the pyrolysis-GC unit = 0.25% by weight. Representative kerogen types: Ip, torbanite (*Botryococcus*) kerogens, rich in alginite (samples 1, 2); Ipn, tasmanite (alginite)-rich kerogens (sample 3); In, resinite-derived materials (sample 4); IIpn, amorphinite-rich kerogens from source rocks containing marine organic matter (samples 5–11); II/IIp, exinite-rich kerogens (samples 12–16); II/IIpp, Carboniferous sporinites (samples 17, 18); IIIpp, vitrinite-rich kerogens from marine/deltaic shales (samples 19–26); IIIap, vitrinite-rich kerogens from coal swamps (samples 27, 28); IV, inertinite-rich kerogens (sample 29). p, Paraffin-dominated pyrogram; pn, paraffin/naphthene-dominated; n, naphthene-dominated; pp, phenol/paraffin-dominated; ap, aromatic hydrocarbon/phenol-dominated.

As described above, immature type III kerogens may have paraffin contents ranging from high (IIIp) to almost zero (IIIap); type IV sediments are characterized solely by lean aromatic hydrocarbon-dominated pyrolysates¹².

Molecular approaches to kerogen typing are still in their infancy, but together with bulk chemical and petrographic characterization methods, they provide an improved method for examining the chemically and physically complex mixtures that represent the mixed type II/III kerogens of many near-shore marine settings. It is these mixed assemblages that are the least well-defined using established approaches, yet are the most common organic facies found in many of the world's basins currently being explored. The general approach outlined here,

while far from resolving this critical problem, is believed to be a necessary step in the development of new high-resolution quantitative methods for source rock kerogen characterization.

We thank Dr Bruce Bromley for assistance with data acquisition software and Unocal management for agreement to publish these data. John Brown assisted with manuscript review.

Received 22 July; accepted 18 September 1985.

1. Tissot, B. P. & Welte, D. H. *Petroleum Formation and Occurrence* (Springer, Berlin, 1978).
2. Gutjahr, C. C. M. *Geologie Mijnb.* 62, 417-425 (1983).
3. Tissot, B. P., Durand, B., Espitalie, J. & Combaz, A. *Bull. Am. Ass. Petrol. Geol.* 58, 499 (1974).
4. Van Krevelen, D. W. *Org. Geochem.* 6, 1-10 (1984).
5. Larter, S. R. in *Organic Geochemistry in Exploration of the Norwegian Shelf* (ed. Thomas, B. M.) (Norsk. Petrol. Forening Spec. Publ., in the press).
6. Philp, R. P. & Gilbert, T. D. *Org. Geochem.* 6, 489-503 (1984).
7. Larter, S. R., Solli, H., Douglas, A. G., DeLange, F. & DeLeeuw, J. W. *Nature* 279, 405 (1979).
8. Van de Meent, D., Brown, S. C., Philp, R. P. & Simoneit, B. R. T. *Geochim. cosmochim. Acta* 44, 999 (1980).
9. Van Graas, G., DeLeeuw, J. W. & Schenk, P. A. *Geochim. cosmochim. Acta* 45, 2465 (1981).
10. Giraud, A. *Bull. Am. Petrol. Geol.* 54, 439 (1970).
11. Larter, S. R. & Douglas, A. G. *J. analyt. appl. Pyrolysis* 4, 1-19 (1982).
12. Larter, S. R. in *Analytical Pyrolysis—Methods and Applications* (ed. Voorhees, K.) 212-275 (Butterworths, London, 1984).
13. Horsfield, B. in *Advances in Petroleum Geochemistry* Vol. 1 (eds Brooks, J. & Welte, D. H.) 247-298 (Academic, London, 1984).
14. Gormly, J. R. & Mukhopadhyay, P. K. in *Advances in Organic Geochemistry 1981* (eds Bjoroy, M. et al.) 597-606 (Wiley, London, 1983).
15. Bracewell, J. M. & Robertson, G. W. *J. analyt. appl. Pyrolysis* 6, 19-29 (1984).
16. Orr, W. L. in *Advances in Organic Geochemistry 1981* (eds Bjoroy, M. et al.) 775-788 (Wiley, London, 1983).
17. Senftle, J.-T., Brown, J. H. & Larter, S. R. *Int. J. Coal Geol.* (in the press).

Magnetostratigraphy and palynology demonstrate at least 4 million years of Arctic Ocean sedimentation

A. E. Aksu* & P. J. Mudie†

* Department of Earth Sciences, Memorial University of Newfoundland, St John's, Newfoundland, Canada A1B 3X5

† Geological Survey of Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada B2Y 4A2

It has long been recognized^{1,2} that the Arctic Ocean has a major role in regulating the timing of Northern Hemisphere glaciations, global heat exchange and ocean circulation. Several recent studies³⁻⁷ have attempted to interpret the palaeoclimatic record in sediment cores from the Arctic Ocean, but conflicting ages have been reported for palaeoclimatic events dated by different methods³⁻⁶. To establish a consistent chronology for cores from the central Arctic Ocean, we performed detailed palaeomagnetic and palynological studies of three cores collected⁷ near the crest of the Alpha Ridge, and obtained radiocarbon dates from planktonic foraminifers isolated from 1-cm-thick intervals. Magnetic inclination values for these cores clearly record normal and reversed-polarity intervals that apparently correspond to the Brunhes and Matuyama magnetochrons; the longest core also includes polarity intervals typical of the Gauss and Gilbert magnetochrons. This chronology is confirmed by the first and last occurrences of pollen and dinoflagellate species that can be correlated with European^{8,9} and DSDP¹⁰⁻¹² chronostratigraphies dated by standard biochronological methods. Micromass radiocarbon dates for foraminifers indicate a sedimentation rate of ~1 mm per 1,000 yr. We believe that these data confirm a long (>4 Myr) chronology for the Alpha Ridge cores, the lithostratigraphy of which can be correlated over extensive areas of the western Arctic Ocean²⁻⁷, and more important, provide dinoflagellate and palynomorph data that indicate the validity of early chronology.

Previous chronostratigraphic studies of Arctic Ocean sediments have been hampered by (1) problems of core handling and storage prior to sampling⁶; (2) incomplete reporting of magnetic data^{3,5,6}; (3) lack of dateable calcareous and siliceous microfossils in the tertiary sediments⁵; and (4) uncertain validity of U/Th dates from hemipelagic muds³.

Our cores were obtained in conjunction with high-resolution seismic profiles from the 1983 CESAR ice station⁷ and they

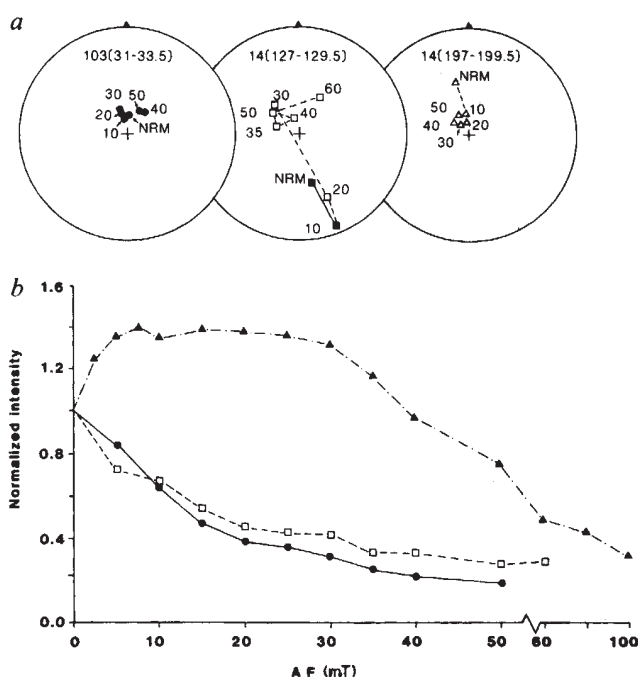


Fig. 1 Examples of directional response to AF demagnetization; open symbols indicate negative inclination, closed symbols indicate positive inclinations (a) and the normalized intensity response to AF demagnetization of these samples (b) where ● = 103 (31-31.5 cm), □ = 14 (127-129.5 cm), △ = 14 (197-199.5 cm).

were stored upright and prevented from freezing before laboratory studies. Two gravity cores, CESAR 102 (85°38.1' N, 111°07.1' W) and CESAR 103 (85°38.1' N, 111°07.0' W), and one long piston core, CESAR 14 (85°50.8' N, 108°21.6' W) were selected for study to ensure that a complete sequence of the surface sediments would be sampled (piston cores rarely recover the surface sediment layer). Cores were split 2-6 months after their recovery, and the sediments are free of post-coring disturbances due to desiccation, oxidation and previous sampling. Palaeomagnetic samples were taken using a plastic cylinder of 2.5 cm diameter¹³. After palaeomagnetic measurement, each sample was split at right angles to the vertical core axis, and the sediments were processed for palynological studies using HCl, HF and sieving methods¹⁴.

Palaeomagnetic measurements of the samples were made¹³⁻¹⁵ using a Schonstedt DSM-1 Digital Spinner Magnetometer. The intensity of the natural remanent magnetization (NRM) ranged from 0.1×10^{-2} A m⁻¹ in normally magnetized samples to 0.5×10^{-3} A m⁻¹ in reversely magnetized samples. Further magnetization measurements were made after alternating field (AF) demagnetization steps until the intensity values were reduced to <15% of the NRM, which usually occurred between 30 and 40 mT. As a precaution against excess demagnetization, however, all samples were stepwise demagnetized at 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35 and 40 mT, with a few samples further demagnetized to 100 mT. Most normal and reverse stable remanence directions had associated precision parameters, k , with corresponding semi-cones of confidence, α_{95} , being less than 5°.

Figure 1 shows examples of the normalized intensity and directional response to AF demagnetization. Some of the remanent magnetization directional scatter may be caused by using the 'push-in' sampling method¹⁶. Normally magnetized core sections typically showed a steady decrease in intensity with demagnetization, accompanied by little directional response. Reversely magnetized sections typically showed an initial positive NRM that gradually changed to negative values with removal of the overprinted viscous remanent magnetization (VRM) after treatment at 10-20 mT. The steady decrease in normalized intensity suggested that the reverse stable remanence

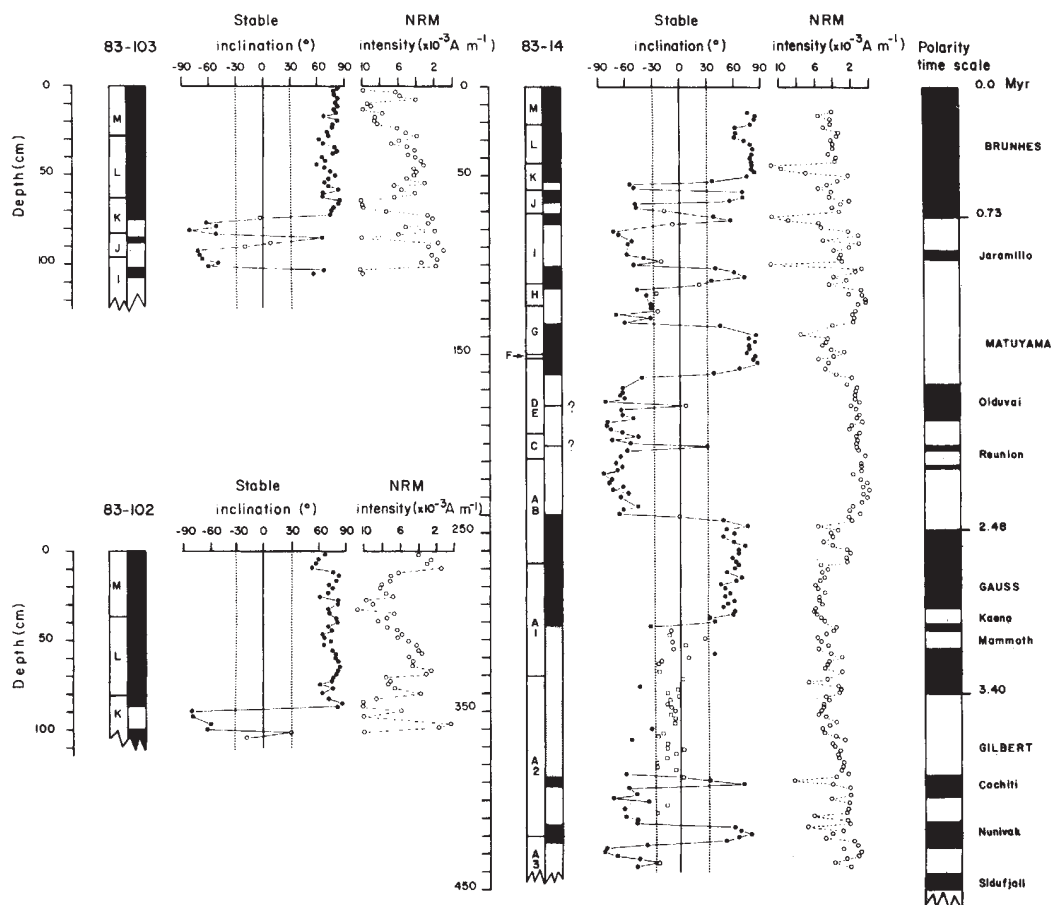


Fig. 2 Downcore plots of stable inclinations (●) and NRM intensities (○). ○, Transition zones; □, samples in which a stable phase of magnetization was not achieved. A3-M, lithostratigraphic units⁷. Summary polarity logs for each core are based on all polarity changes that exceed +30 or -30° on their stable inclination; values of <30° are indicated as transitional. Polarity time-scale is from Harland *et al.*¹⁸.

intensity was weaker than the VRM. Some normally magnetized samples and about 20% of the reversely magnetized samples showed a progressive increase of intensity on treatment at 10 or 15 mT, followed by a steady decrease on demagnetization at 50 or 100 mT. Removal of the VRM caused little directional response in these samples.

Plots of stable inclination versus depth (Fig. 2) clearly reveal normal and reversed-polarity chrons like those observed in global magnetostratigraphies^{17,18}. The Brunhes-Matuyama boundary occurs around 71-77 cm depth in CESAR 103, at 85-90 cm in CESAR 102 and at 50-55 cm in CESAR 14. In all cores, this transition is sharp and is associated with a decrease in intensity of $0.8 \times 10^{-3} \text{ A m}^{-1}$ in the middle of lithofacies K. The Matuyama-Gauss transition is identified from the 239-244 cm depth in CESAR 14. Within the Matuyama reverse chron, four major positive subchrons occur in CESAR 14 and two are found in the gravity cores. All polarity changes are associated with large changes in intensity. The most prominent subchron (135-161 cm in CESAR 14) is tentatively correlated with the Olduvai normal event. The two short-lived positive events just below the Brunhes-Matuyama boundary are tentatively correlated with the Jaramillo event. The event at 102-109 cm depth in CESAR 14 may be either a split Olduvai subchron, with the lower part occurring at 135-161 cm, or the Gilsa event. The transition from Gauss normal to Gilbert reverse-polarity chrons is inconspicuous in CESAR 14. The stable inclination oscillated between 60° and 75°, then a 70° shift occurred, below which few samples showed clear stable remanence directions. The Gauss-Gilbert transition is therefore tentatively placed at ~300 cm depth.

Inclination values for the Brunhes and Gauss positive polarity chrons in CESAR 14 (Fig. 3) were strongly skewed towards high positive NRM values and showed little displacement with stepwise demagnetization. NRM inclinations in the reversed polarity Matuyama chron showed a slight skewness towards positive

NRM values, gradually displacing towards the negative values to form a well-defined peak at -45° to -75° by 40 mT. This behaviour demonstrated the successful removal of low-coercivity downward normal components, and the isolation of high-coercivity reversed-polarity components. It is clear that the viscous component is not totally removed before 30-40 mT demagnetization steps, and that samples show progressive directional response with each demagnetization step (Figs 1, 3). In most previous palaeomagnetic studies¹⁹⁻²¹ of Arctic Ocean sediments, samples were routinely demagnetized at 5-15 mT. The CESAR cores show that over 80% of the samples required demagnetization to 40 mT. Hence, insufficient demagnetization may account for the considerable scatter observed in inclination values reported previously.

Palynological studies of CESAR 103 and 14 were made on the samples used for palaeomagnetism measurements. Detailed data for CESAR 103 and initial results for CESAR 14 are documented elsewhere²²; systematic descriptions of the dinocysts (dinoflagellate cysts) are given by Mudie¹¹. The lithostratigraphy (facies A3 to M) and regional core correlations are documented elsewhere⁷. Here (Fig. 4) we show only the ranges of selected dinocyst, pollen and spore taxa in CESAR 14. Some notable similarities between the ranges of the marine dinocysts and those of terrestrial pollen and spores are evident: (1) The base of facies A2 is marked by the last appearance datum (LAD) or disappearance of two dinocyst taxa and five pollen/spore taxa, and one dinocyst taxon and one pollen taxon have their first appearance datum (FAD) in this interval. (2) Between the top of facies AB and C, three dinocysts and one pollen taxa have their LAD. (3) Between the top of facies G and the base of I, one dinocyst taxon disappears, and six dinocyst species and three pollen/spore taxa appear or reappear; the base of the Gramineae/Cyperaceae acme zone is found near the top of facies I. (4) In facies J to K, the calcareous dinoflagellate¹¹, *Thoracosphaera arctica* Gilbert and Clark, and several

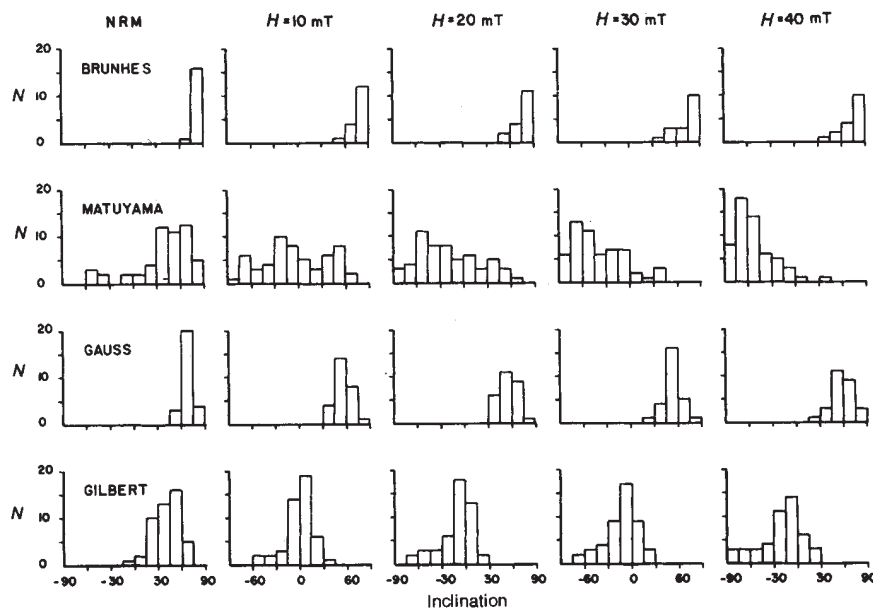


Fig. 3 Histograms of inclination values from major magnetic polarity chrons for CESAR 14; *N*, number of samples. Brunhes = 0–52 cm; Matuyama = 52–240 cm; Gauss = 240–300 cm; Gilbert = 300–450 cm. *H* indicates strength of the AF demagnetization field.

thermophilous tree pollen (*Carya*, *Nyssa* and *Pseudotsuga*) have their LAD, and three dinocysts have their FAD. (5) Facies L marks the LAD of the remaining thermophilous tree pollen taxa, *Juglans*, *Quercus*, *Acer* and *Tsuga*.

The stratigraphic distributions of dinocysts, pollen and spores shown in Fig. 4 can be correlated with DSDP data from the Bering¹⁰ and Norwegian seas¹² and from Site 611 in the North Atlantic south of Greenland¹¹, and with stratotypes in north-west Europe^{8,9} and offshore eastern Canada²³. Palynomorphs from the DSDP sites have been dated by correlation with standard calcareous nannoplankton or diatom zones and by magneto-chronology (Site 611), and the European stratotypes have been dated by various methods⁹, including with DSDP bio-chronologies.

The following ages are thus determined for intervals (1) to (5) listed above.

(1) **Base of A₂.** At DSDP Site 611, the LAD of *Platycystidia* (?) sp. 1 and the FAD of *Brigantedinium* sp. occurs at the base of the Nunivak magnetochron (4.26 Myr). In the Bering Sea, *Oper-*

culodinium centrocarpum, *Alnus* sp. 1, *Picea* sp. 1 and *Gleichenia* disappear in the late Miocene, but *Liquidambar* and *Cedrus* have a late Pliocene LAD. Off eastern Canada, the FAD of *Artemisia* marks the base of the Pliocene. Overall, then, it is clear that facies A₂ is at least late Pliocene in age, and the weight of evidence indicates a middle early Pliocene age which is consistent with the magneto-chronology in Fig. 2.

(2) **Upper AB to C.** At Site 611, *Brigantedinium simplex* has its FAD at the top of the Kaena magnetochron (2.92 Myr), *Operculodinium* sp. of Piasecki has its LAD at the top of the Gauss (2.48 Myr), and in Europe, *Podocarpus* has its LAD at the Reuverian/Praetiglian boundary (2.40 Myr). These ages are in good agreement with our magneto-chronology, although *Impletosphaeridium* sp. and *Filispheera lifera* disappear at the Plio-Pleistocene boundary at Site 611 and in the Bering Sea, possibly indicating a slightly younger age.

(3) **Top of G to base of I.** In the Bering Sea, *Impagidinium pallidum* disappears at the Plio-Pleistocene boundary, and *Multispinula minuta* and *Quinquecupis concreta* have their FAD

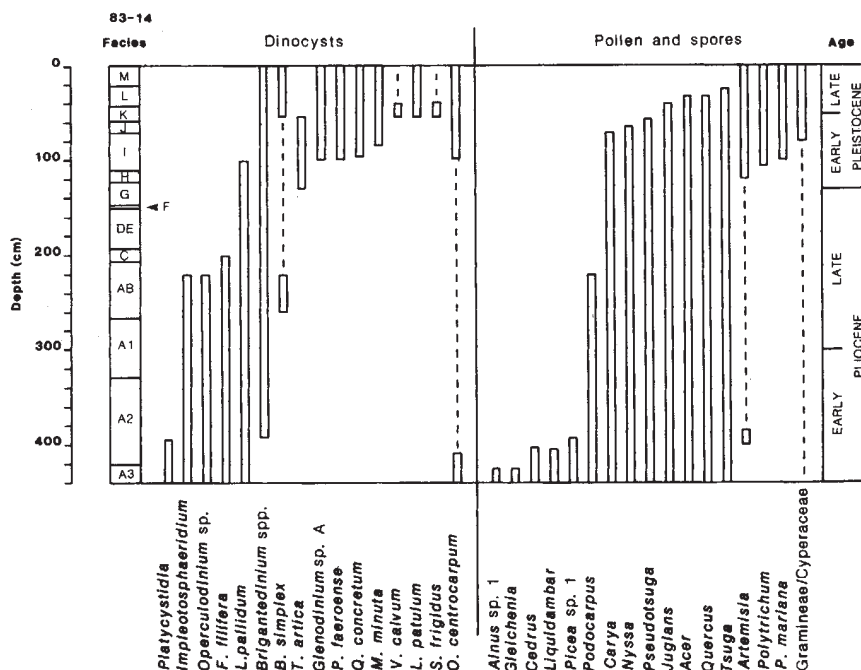


Fig. 4 Stratigraphic ranges of palynomorphs in CESAR 14, compared with the magneto-chronology shown in Fig. 2.

in the early Pleistocene. These occurrences are in accord with our magnetochronology (Fig. 2). No FAD have been reported previously for *Picea mariana*, *Polytrichum* or *Peridinium faeroense* Dale, but at Site 611, these boreal taxa first become common at the onset of cooling at the Plio-Pleistocene boundary. The range of *T. arctica* has been dated as early Pleistocene in a number of other Arctic Ocean cores^{9,24} using palaeomagnetism and U/Th dating methods, and *O. centrocarpum* has an early Pleistocene acme zone in the Bering Sea.

(4) **Facies J to K.** At Site 611 and in the Bering Sea, *Spiniferites frigidus* has its FAD at or near the early/late Pleistocene boundary, which agrees well with our data for CESAR 14. In contrast, the FAD of *Votadinium calvum* and *Impagidinium patulum* and the base of the Gramineae/Cyperaceae acme zone occurs in the late Pliocene or earlier in the North Atlantic, *Nyssa* has its LAD in the late Pliocene of Europe and eastern Canada, and *Pseudotsuga* disappears at the top of the Cromerian Stage (~1 Myr) in Europe. Overall, correlation is not very precise for this interval in CESAR 14, but the palynomorphs definitely indicate a minimum middle Pleistocene age rather than the shorter (0.2 Myr) chronology proposed by Sejrup *et al.*³.

(5) **Facies L.** In Europe, *Juglans* has its LAD at the Holstein/Saalian boundary (~0.5 Myr), and Site 611, the tops of the *Quercus* and *Acer* acmes, and the LAD of *Tsuga* occur in the middle Pleistocene, indicating an age of 0.5 to at least 0.2 Myr, which agrees reasonably well with the magnetochronology proposed for CESAR 14.

Palynological correlation thus provides a chronology for the Alpha Ridge cores which generally supports the magnetochronology shown in Fig. 2. Hence, we conclude that the sedimentation rate on the Alpha Ridge has been very slow, about 1–1.5 mm per 1,000 yr, during the past 4.25 Myr. Further evidence for a very slow sedimentation rate is provided by tandem accelerator micromass radiocarbon dates from planktonic foraminifers (*Neoglobobulimina pachyderma* sinistral) that were hand-picked from the >150- μ m sediment fraction of CESAR 102 at 2–3 cm and 5–6 cm depth. Dates of >30,000 yr BP were obtained for both these samples (laboratory numbers Beta-12230 and Beta-12231, respectively). This slow deposition rate is in accord with previous magnetostratigraphic studies^{2,5,9,19,21}. These data conflict strongly with the short chronology and higher sedimentation rates (1–2 cm per 1,000 yr) obtained from dating by amino-acid epimerization³. In view of the potential importance of understanding the role of the Arctic Ocean in regulating the timing of Northern Hemisphere glaciations^{1,2}, detailed magnetostratigraphic and lithostratigraphic correlations and confirmation of dating using organic walled palynomorphs (which are not susceptible to carbonate or silica dissolution and are thus present in all the lithofacies) are important prerequisites for studying palaeoecological and isotope records from Arctic Ocean cores.

We thank C. Walls, D. C. Younger, L. Dobbin, K. McInnon and L. Ogden (Dalhousie University) for sediment sampling and preparation for laboratory measurements. The study was supported by DSS contract OSC83-00399 to Dalhousie University, and by GSC support for Project 820044. We thank S. M. Blasco and R. Fensome of the Atlantic Geoscience Centre, and Edward Irving for critical comments.

Received 20 June; accepted 29 August 1985.

- Ewing, M. & Donn, W. L. *Science* **123**, 1061–1066 (1956).
- Clark, D. L. *Bull. geol. Soc. Am.* **82**, 3313–3323 (1971).
- Sejrup, H. P., Miller, G. H., Brigham-Grette, J., Lovlie, R. & Hopkins, D. *Nature* **310**, 772–775 (1984).
- Zahn, R., Markus, B. & Thiede, J. *Nature* **314**, 433–435 (1985).
- Worsley, T. R. & Herman, Y. *Science* **210**, 323–325 (1980).
- Clark, D. L. *Nature* **300**, 321–325 (1982).
- Mudie, P. J. & Blasco, S. M. *Geol. Surv. Can. Pap.* **84-22**, 59–100 (1985).
- Van der Hammen, T., Wijmstra, T. A. & Zagwijn, W. H. in *The Late Cenozoic Ages* (ed. Turekian, K. K.) 391–424 (Yale University Press, 1971).
- Suc, J. & Zagwijn, W. H. *Boreas* **12**, 153–166 (1983).
- Bujak, J. P. *Micropaleontology* **30**, 180–212 (1984).
- Mudie, P. J. in *Init. Rep. DSDP* **94** (in the press).
- Koreneva, E. V., Zaklinskaya, E. D., Bratseva, G. M. & Kertashova, G. G. *Init. Rep. DSDP* **38**, 1169–1193 (1976).
- Aksu, A. E. *Geol. Surv. Can. Pap.* **84-22**, 101–114 (1985).

- Mudie, P. J. *Can. J. Earth Sci.* **19**, 729–747 (1982).
- Aksu, A. E. *Geology* **11**, 537–541 (1983).
- Gravenor, C. P., Symons, D. T. A. & Coyle, D. A. *Geophys. Res. Lett.* **1**, 836–839 (1984).
- Mankinen, E. A. & Dalrymple, G. B. *J. geophys. Res.* **84**, 615–626 (1979).
- Harland, W. B. *et al. Geological Time Scale* (Cambridge University Press, 1982).
- Steuerwald, B. A., Clark, D. L. & Andrew, J. T. *Earth planet. Sci. Lett.* **5**, 79–85 (1968).
- Clark, D. L. *Bull. geol. Soc. Am.* **81**, 3129–3134 (1970).
- Hunkins, K., Be, A. W. H., Opdyke, N. D. & Mathieu, G. *The Late Cenozoic Ages* (ed. Turekian, K. K.) 215–237 (Yale University Press, 1971).
- Mudie, P. J. *Geol. Surv. Can. Pap.* **84-22**, 149–174 (1985).
- Williams, G. L. & Bujak, J. P. *Am. Ass. Stratigr. Palynol. Contr. Ser. No. 5A*, 14–47 (1977).
- Gilbert, M. W. & Clark, D. L. *Mar. Micropaleont.* **7**, 385–401 (1983).

Fronde-like fossils from the base of the late Precambrian Wilpena Group, South Australia

Ian A. Dyson

School of Earth Sciences, Flinders University of South Australia, Sturt Road, Bedford Park, South Australia 5042, Australia

Late Precambrian metazoan fossils are at present known from at least 23 different regions in Africa, Asia, Australia, Europe and North America¹. The Ediacara fossil assemblage^{2,3} occurs widely in the Flinders Ranges, South Australia, near the base of the Rawnsley quartzite of the Pound Subgroup. This complex of soft-bodied organisms includes medusoid coelenterates, soft corals related to pennatulids, segmented worms, arthropods and other simple coelenterates^{4,5}. Here I report the discovery of frond-like fossils comparable to those of the Ediacara assemblage more than 3,000 m stratigraphically below the Ediacara Member⁶ of the Pound Subgroup. This discovery supports the placement of the base of the Ediacaran⁵ in South Australia at the boundary of the Wilpena Group and the Umberatana Group, a stratigraphic level which marks the termination of late Proterozoic glaciation in the Adelaide Geosyncline.

The Adelaide Geosyncline (Fig. 1) represents a sedimentary sequence that accumulated in response to a branching network of separate rifts with an overall meridional trend across the middle of the Australian continent^{7,8}. The late Precambrian Wilpena Group, a succession of predominantly terrigenous clastics deposited within the Adelaide geosyncline^{9,10}, rests conformably on glaciogenic sediments of the Umberatana Group¹¹. The Brachina Subgroup is the lower of two upward-coarsening sequences within the Wilpena Group (Fig. 2). A detailed stratigraphic study of this sequence at Hallett Cove, South Australia (Fig. 1), has identified turbidite and storm-dominated shallow marine facies¹². This section correlates very strongly with that of the fossil site.

The fossils reported here are preserved on an upper bedding-plane surface within basal sandstones of the Brachina Subgroup cropping out in Alligator Gorge of the Mount Remarkable National Park in the southern Flinders Ranges (Fig. 1). This sequence passes upward from massive sandstones which inter-tongue with dolomites of the Nuccaleena Formation, through interbedded shales and fine sandstones, into a thick medium-grained cross-bedded unit known as the ABC Range quartzite (Fig. 2). The upward coarsening and thickening Brachina Subgroup is interpreted to be the result of initial transgression followed by progradation into a sedimentary basin. Sedimentation took place in a storm- and tide-dominated shallow marine environment with an ENE slope into a deeper turbidite basin.

I do not propose to present a detailed taxonomic classification of the metazoan fossil. However, further investigation of the study area will hopefully result in the discovery of more specimens to help with systematics (the specimen has not been removed from the discovery site). The novel fossil may be interpreted as representing organisms which were basically foliate, leaf-shaped (frond) structures, with each preserved specimen lying on one of its two broad sides on a bedding plane within the sandstone. The frond-like structures consist of a

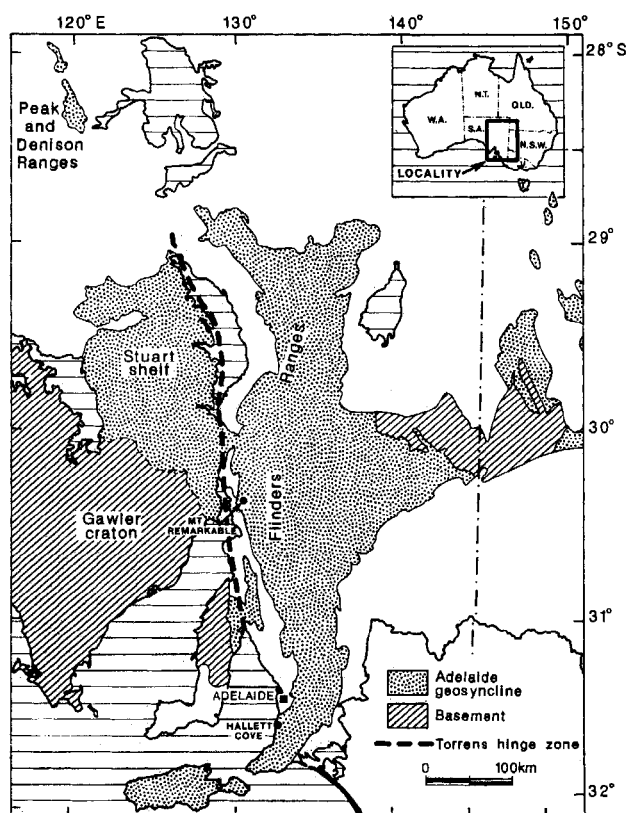


Fig. 1 Outcrop area of the late Proterozoic Adelaide geosyncline. Note the locality of the study area: Mount Remarkable in the southern Flinders Ranges.

relatively narrow median zone from which extend bilaterally opposed leaf-like branches. The specimens show regular spacing of branches, with specimen *b* (Fig. 3) displaying a wider spacing between consecutive branches. A number of incomplete individuals, preserved as impressions on a very thin shale parting in massive sandstone, are thought to have been oriented by rip or turbidity currents flowing in an ENE direction. The specimens overlap and show signs of distortion, making identification of individual outlines difficult. An incomplete frond (a in Fig. 3) is ~30 cm long and 6.5 cm wide, and is composed of about 30 partially or completely preserved branches extending from one side of a broad axial region. The median track is ~20 mm wide and tapers to a point at one end. Large areas of the branches show no systematic curvature, with each branch extending from the median track at an angle of 45°. Each branch is bounded by a well-defined groove. A more complete specimen (b in Fig. 3) clearly exhibits a narrower median track about which numerous lateral branches exhibit bilateral symmetry. The branches appear to extend opposite one another at an angle of 70–85° from the median track and are bounded by evenly spaced repetitive grooves. The specimen is 37 cm long and 9 cm wide.

Comparison of these specimens with those fossils from the Pound Subgroup representing sessile colonial *Cnidaria*¹ indicates that the metazoans of this latest discovery in the Flinders Ranges may belong to one of the two families Pteridiniidae Richter, 1955 and Charniidae Glaessner, 1979, as the fossils consist of a median track from which a leaf-like expansion extends with bilateral symmetry. Members of the former family are rare from the Flinders Ranges. These frond-like fossils of the Ediacara assemblage are considered to be allied to the living members of the Order Pennatulacea^{1,13}. Modern pennatulids (sea pens) are found growing vertically on sandy sea floors¹⁴ with their stalk moored to the substrate by a holdfast. Sea pens are found washed up on beaches, after being torn from their moorings by storm action. Fossils of the genus *Pteridinium*

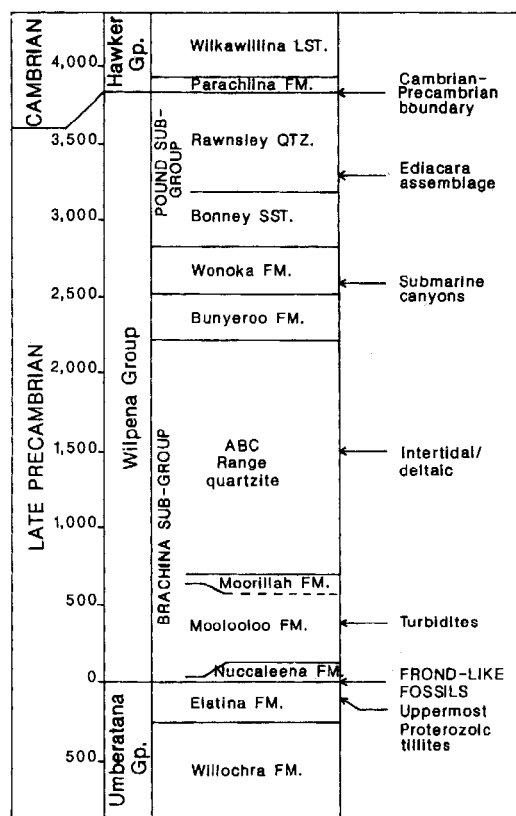


Fig. 2 Stratigraphy of the late Precambrian Wilpena Group in southern Flinders Ranges. LST., limestone; FM., formation; QTZ., quartzite; SST., sandstone.

Gürich are known to occur in storm beds¹⁵. The median track of *Charniodiscus* Ford² extends downwards into a stalk which terminates in a basal attachment disk; lateral branches extend from the median track at an inclination of ~65–85° and bear foliate structures (polyp leaves) divided into ridges (polyp anthostoles). These secondary divisions that occur between the polyp anthostoles of the Charniidae, appear to be rare and minute in the family Pteridiniidae¹. In *Pteridinium*, three ribbed wings may extend from the axis¹⁶. *Pteridinium simplex* is characterized by its incised zig-zag median line and evenly spaced, repetitive lateral grooves which extend from the axis at ~70°. No stalks or attachment disks are known¹ for this species.

Strong similarities exist between the Brachina Subgroup fossils and *Pteridinium* found in the late Precambrian sedimentary units of the Nama Group of south-west Africa^{16–18}, the Valdai sediments of the White Sea coast of the USSR^{18–20}, the Carolina slate belt of the southeastern United States²¹, and the Pound Subgroup of the Ediacara Hills, South Australia^{13,22}. The Brachina Subgroup fossils are comparable in size and morphology to those of the Ediacara assemblage. There is no evidence of secondary grooving, as occurs between the anthostoles of the Charniidae, nor of any anchoring device, therefore I provisionally assign the fossils to the genus *Pteridinium* Gürich.

The Ediacara assemblage from the Pound Subgroup (Fig. 2) has been assigned a late Precambrian age^{1,3}, as have similar fossils from various localities around the world. The proposed Ediacaran Period^{5,23}, during which metazoans appear to have become diverse and relatively numerous, has been assigned to the interval 670–550 Myr⁴. In the present study area, the base of the Wilpena Group lies more than 3,000 m stratigraphically below the Ediacara Member of the Pound Subgroup (Fig. 2). The base of the Wonoka Formation (Fig. 2) has been suggested as the lower boundary of the Ediacaran in South Australia⁵. The first early metazoan assemblages were thought to appear at

Fig. 3 Photograph of the frond-like fossils preserved as impressions on thin shale parting in massive sandstone. Specimens *a* and *b* are described in the text. The coin, shown for scale, is 24 mm in diameter.



~640–620 Myr, and became diverse and frequent during the interval ~600–570 Myr. Ediacara assemblages should comprise almost by definition the first occurrences of metazoan body fossils^{1,4}. Radiometric dating suggests an age of 676 ± 204 Myr for the Brachina Subgroup west of the Flinders Ranges²⁴. The discovery of fossils in the Brachina Subgroup that are similar in size to those of the Ediacara assemblage, supports the placement of the lower boundary of the Ediacaran Period at the base of the Wilpena Group⁴. In addition, the fossils are the first unequivocal metazoan remains to be found in the Brachina Subgroup.

The discovery of metazoans in the Brachina Subgroup raises questions concerning radiometric ages and worldwide stratigraphic correlations. This sedimentary unit rests conformably on the Umberatana Group which marks the termination of late Proterozoic glaciation in the Adelaide geosyncline. An event which in several major regions pre-dates the first appearance of Metazoa is the last occurrence of Precambrian tillites¹. Such tillites overlain by Ediacara assemblages are known in Australia, south-west Africa, China, eastern Europe, Newfoundland, North Carolina and Sweden and may provide the potential for global lithological and biostratigraphical correlations. However, the recording of these various first appearances needs to be addressed further in stratigraphic and geochronometric terms.

Financial support was provided by the Flinders University research budget. I thank Bridget Youngs, Chris von der Borch and Alex Grady who initially reviewed the manuscript and provided many helpful comments. The figures were drafted by Gail Jackson, and Libby Eatts assisted with typing. Karin Sokoll and Alanah Sexton provided valuable field and research support respectively. I particularly thank Jim Gehling and Martin Glaessner for stimulating discussions during various stages of this work.

Received 13 February; accepted 20 September 1985.

- Glaessner, M. F. *The Dawn of Animal Life* (Cambridge University Press, 1984).
- Jenkins, R. J. F., Ford, C. & Gehling, J. G. *J. geol. Soc. Aust.* **30**, 101–119 (1983).
- Jenkins, R. J. F. & Gehling, J. G. *Rec. S. Aust. Mus.* **17**, 347–359 (1978).
- Glaessner, M. F. *Bull. geol. Soc. Am.* **82**, 509–514 (1971).
- Cloud, P. & Glaessner, M. F. *Science* **217**, 783–792 (1982).
- Jenkins, R. J. F. *Trans. R. Soc. S. Aust.* **105**, 179–194 (1981).
- Preis, W. F., Rutland, R. W. & Murrell, B. in *Precambrian of the Southern Hemisphere: Developments in Precambrian Geology Vol. 2* (ed. Hunter, D. R.) 327–360 (Elsevier, Amsterdam, 1981).
- von der Borch, C. C. *Tectonophysics* **70**, 115–134 (1980).
- Thompson, B. P. in *Handbook of South Australia Geology* (ed. Parkin, L. W.) 49–83 (Geol. Surv. S. Aust., Govt. Printer, Adelaide, 1969).
- Thompson, B. P. *Trans. R. Soc. S. Aust.* **94**, 193–221 (1970).
- Coats, B. P. *Q. Notes geol. Surv. S. Aust.* **11**, 1–2 (1964).
- Dyson, I. A. *Geol. Soc. Aust. Abstr.* **10**, 49–50 (1983).
- Glaessner, M. F. & Wade, M. *Palaeontology* **9**, 599–628 (1966).
- Attenborough, D. *Life on Earth* (Collins, London, 1979).
- Wade, M. *Proc. Soc. Vic.* **84**, 183–189 (1972).

- Pflug, H. D. *Palaeontographica* **134**, 226–262 (1970).
- Richter, R. *Senckenbergiana lethaea* **36**, 234–289 (1955).
- Germis, J. B. *Bull. Chamber of Mines Precamb. Res. Unit* **12**, 250p (Univ. Cape Town Dept Geol., 1972).
- Fedonkin, M. A. *Dokl. Akad. Nauk. SSSR* **239**, 1423–1426 (1978).
- Fedonkin, M. A. *Palaeont. Zhurnal* **2**, 107–115 (1980).
- Fedonkin, M. A. *Trans. Akad. Nauk. SSSR, Geol. Inst.* **342**, 1–100 (1981).
- Gibson, G. G., Teeter, S. A. & Fedonkin, M. A. *Geology* **12**, 387–390 (1984).
- Glaessner, M. F. *Annln naturh. Mus. Wein* **66**, 113–120 (1963).
- Coats, R. P. & Preiss, W. V. *Precamb. Res.* **12**, 181–208 (1980).

Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*

S. M. Assmann*, L. Simoncini† & J. I. Schroeder†

* Department of Biological Sciences, Stanford University, Stanford, California 94305, USA

† Department of Membrane Biophysics, Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, FRG

Transmembrane ion exchange and blue-light dependent responses are central processes in plants in general^{1,2} and stomatal guard cells in particular³. Stimuli, including light, cause H^+ extrusion by stomatal guard cells^{4,5}, K^+ uptake, osmotic swelling and enlarged stomatal pore apertures, resulting in increased leaf conductance for CO_2 influx and water efflux⁶. The mechanisms underlying ion movement and the specific response of stomata to blue light^{7–9} remain, however, unknown. We have now investigated these phenomena using gas-exchange techniques on leaves and whole-cell patch clamping^{10,11} of protoplasts. Blue light-stimulated stomatal opening in the leaf is correlated with activation of an electrogenic pump in the guard cell plasmalemma which results in hyperpolarizations as large as 45 mV and outward currents as large as 5.5 pA. The pump functions in the absence of K^+ ; in the presence of K^+ an increase in voltage noise upon light-induced hyperpolarization more negative than the K^+ equilibrium potential implies K^+ flow through K^+ -selective channels¹². The data are in accord with a chemiosmotic model of stomatal response¹³ whereby active H^+ extrusion creates an electrochemical gradient for passive ion fluxes. A 25–35-s delay in the onset of hyperpolarization, and its peak several minutes after application of a blue light pulse suggest that blue light functions as an activator, rather than a direct energy source, for ion pumping.

Figure 1 shows a typical change in hydraulic conductivity, commonly referred to as 'stomatal conductance' of a leaf of *Vicia faba* L. (cv. long pod) following application of a transient blue light stimulus under a constant background illumination

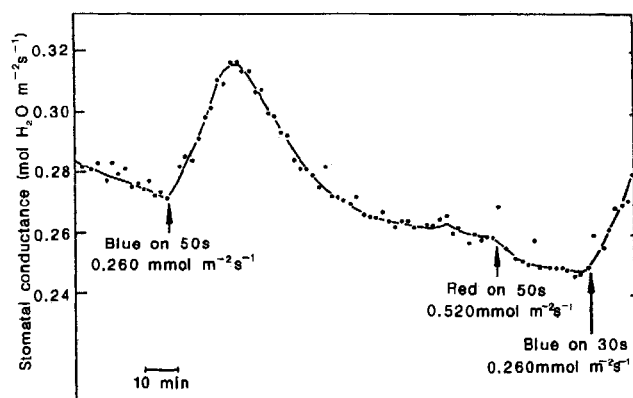


Fig. 1 Transient increase in stomatal conductance in response to a 50-s pulse of $0.260 \text{ mmol m}^{-2} \text{ s}^{-1}$ incident blue light given under $0.525 \text{ mmol m}^{-2} \text{ s}^{-1}$ background red light. Note the absence of any increase in conductance following a 50-s pulse of $0.520 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light applied in the same manner. Background red light was provided by a Sylvania 300W (PAR56/2MEL) lamp used in conjunction with a Kodak 1A red filter (50% cut-off at 645 nm) and a layer of No. 5A Cinemoid. Pulsed light was obtained by filtering light from a GE Gemini 300 projector lamp through a wideband hot mirror (OCLI, Santa Rosa, California) and a Roehm and Haas 2424 blue Plexiglass filter (maximum transmittance at 470 nm, half-bandwidth 100 nm) or a Corning 2-61 red glass filter. Stomatal conductance was measured using a null balance gas-exchange system³⁰. Conditions inside the gas-exchange cuvette were maintained at 23 °C, ambient CO_2 $300\text{--}250 \mu\text{l l}^{-1}$, vapour pressure deficit $0.75\text{--}0.95 \text{ kPa}$.

with high fluence rates of red light. As with *Commelina communis*¹⁴, a pulse of blue light results in a transient rise in stomatal conductance to water vapour which peaks ~ 20 min later. A red light pulse produces no increase in conductance (Fig. 1), demonstrating that the response stimulated by blue light is independent of both photosynthesis and the component of the stomatal response to light which is mediated by the guard cell chloroplasts¹⁵. The response can thus be attributed specifically to the action of the guard cell blue light photoreceptor⁷⁻⁹.

The high specificity provided by this dual-beam protocol^{8,14} was further utilized in an electrophysiological investigation of the membrane properties and ion fluxes underlying the blue light response of stomata. Studies using conventional electrophysiological techniques have shown hyperpolarization in response to white light^{16,17}. We used the whole cell patch-clamp technique^{10,11}, instead of intracellular recording, to ensure an unambiguous characterization of electrical responses of the plasmalemma as opposed to the tonoplast or a series combination of the two membranes¹⁸, thus solving a ubiquitous problem in plant electrophysiology. Patch clamping allowed us to observe either membrane potential (V_m) or membrane current, and to define solutions on both sides of the plasmalemma, so that gradients for passive ion fluxes could be manipulated or eliminated.

Seal formation on single guard cell protoplasts of *V. faba* L. (cv. long pod and grünehangdown) was facilitated by the presence of 1 mM CaCl_2 in the bath solution, by a pipette solution with an osmolarity 10% less than that of the bath solution¹⁰, and by the use of Kimax glass (Kimble 34500) pipettes with tip resistances of $10\text{--}20 \text{ M}\Omega$ in symmetrical 150 mM KCl solutions¹⁹. Typical whole-cell membrane resistances were $1\text{--}5 \text{ G}\Omega$, although resistances as high as $20 \text{ G}\Omega$ were observed. Immediately after the establishment of the whole-cell configuration, healthy cells exhibited a V_m of -20 to -50 mV which then declined towards 0 mV , presumably as a result of diffusional equilibration between the cell contents and the pipette solution. Cells typically stabilized at resting potentials of -10 to $+10 \text{ mV}$.

In symmetrical 50 mM K^+ -glutamate solutions, a 30-s pulse of $0.050 \text{ mmol m}^{-2} \text{ s}^{-1}$ blue light given on a background of $0.500 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light resulted in a transient membrane

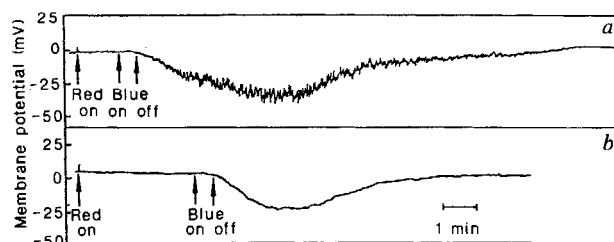


Fig. 2 *a*, Transient membrane hyperpolarization in a guard cell protoplast in whole-cell recording mode, current clamp, in response to a 30-s pulse of $0.050 \text{ mmol m}^{-2} \text{ s}^{-1}$ blue light superimposed on a $0.500 \text{ mmol m}^{-2} \text{ s}^{-1}$ red light background. Incident fluence rates were measured above the air-water interface. Note the increase in voltage noise as the membrane hyperpolarized and the delay before the initiation of the response. Both external and pipette solutions contained 50 mM K^+ -glutamate, 5 mM MgCl_2 , 10 mM HEPES , 3 mM NaOH ; internal solution contained in addition $9.2 \text{ mM (Na}^+)_2\text{-ATP}$ in 20 mM Tris (final concentrations) and $100 \mu\text{M EGTA}$. External solution was $\text{pH } 7.15$, $500 \text{ mosmol kg}^{-1}$; internal solution was $\text{pH } 7.4$, $450 \text{ mosmol kg}^{-1}$. Osmolarities were measured in a vapour pressure osmometer (Wescor 5100c) and adjusted by the addition of D-mannitol. Temperature $22\text{--}24^\circ \text{C}$. *b*, Membrane hyperpolarization in response to blue light. Solutions were the same as in *a* except that K^+ was replaced by the impermeant cation *n*-methylglucamine in both the external and pipette solutions, and the external solution contained 1 mM CaCl_2 . Note the relative absence of voltage noise. *a* and *b* show no response in dark-adapted cells on an initial exposure to the background red light; occasionally cells showed a transient hyperpolarization when the background light was turned on (not illustrated). The following conditions were identical for *a* and *b*. Background red light was obtained by placing a Kodak No. 29 gelatin (50% cut-off at 590 nm) and a Cinemoid No. 5A filter in the microscope light path (Zeiss 12V, 60-W 1251 bulb). Blue light was obtained by filtering light from a Liesegang Fantimat 150Af projector lamp through a Roehm and Haas 2424 blue Plexiglass filter. Control of membrane current and measurement of membrane potential were performed with an EPC-7 patch clamp (List Electronic) and recorded on a Racal store 4Ds tape recorder and a chart recorder. Protoplasts were obtained by modification of a published procedure³¹. Abaxial epidermal peels from six leaves of 3-6-week-old plants were incubated in 15 ml of 2% Cellulase Onozuka RS (Yakult Honsha Co.) and 0.022% Pectolyase Y-23 (Seishin Pharmaceutical Co.), 0.3 M D-mannitol and 1 mM CaCl_2 for 50-65 min in a shaking water bath (temperature $29\text{--}31^\circ \text{C}$). Following passage through a $30\text{-}\mu\text{m}$ mesh, the enzyme solution was centrifuged at $125g$ for 12 min (Sigma 3E centrifuge) and the pellet, containing the guard cell protoplasts, was washed twice ($125g$ for 8 min) in 0.5 M D-mannitol with 1 mM CaCl_2 and stored on ice in the same solution.

hyperpolarization peaking several minutes later (Fig. 2*a*). The characteristics of the hyperpolarization were similar to those of the stomatal response in the whole leaf (compare Figs 1 and 2) but had a faster time course, as might be expected of an early event in the phototransduction process occurring in an isolated system. Patch clamping allowed an accurate resolution of the initial kinetics of the response to blue light, revealing a 25-35-s delay between the onset of the blue light and the beginning of hyperpolarization. An identical delay was observed under longer illuminations with blue light (not illustrated), demonstrating that the onset of hyperpolarization is not correlated with termination of the blue light signal. Similar response kinetics were observed when the major permeant cation, K^+ , was replaced by the impermeant ion *n*-methylglucamine (Fig. 2*b*). In the absence of K^+ , the voltage noise of the response was greatly reduced, suggesting that this noise represents passive flux of K^+ through discrete channels¹² in response to the change in V_m . As in the intact leaf, no such response was observed to a pulse of red light ($n=7$, cf Fig. 3*a*), demonstrating that the responses illustrated in Fig. 2 are mediated by the blue light photosystem.

The blue light response was observed in cells that had resting potentials from -30 to $+10 \text{ mV}$ and within this range exhibited no apparent voltage dependence ($n=20$, not shown). No response to blue light was observed when ATP was eliminated

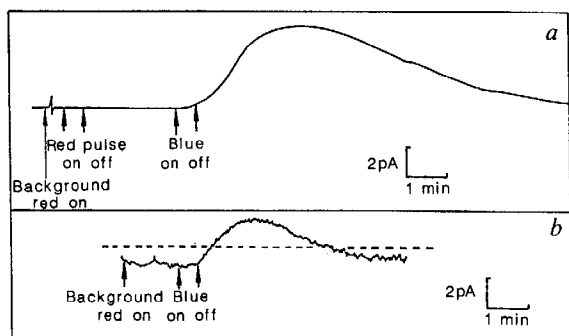


Fig. 3 *a*, Response of whole-cell membrane current to blue light. The membrane potential of the cell was voltage-clamped at $V_m = -4$ mV and the current response was low-pass-filtered at 10 Hz. Smaller current responses were also observed at this voltage (not illustrated). Solutions and illumination protocol were the same as in Fig. 2*b*. Note the absence of a response to a 30-s pulse of 0.050 mmol $m^{-2} s^{-1}$ red light, obtained by transiently increasing the intensity of the microscope light. *b*, Same recording conditions as *a* except that the cell was voltage-clamped at -50 mV and the response was filtered at 60 Hz. Temperature $19^\circ C$. The response exceeds the zero current level (dashed line), indicating that blue light activates an outward current rather than an increase in membrane resistance.

from the pipette solution ($n = 7$, not shown). These observations led us to postulate that the hyperpolarization induced by blue light resulted from the activation of an electrogenic plasmamembrane ATPase. To test this hypothesis, the membrane potential was clamped at -50 mV, a potential more negative than the Nernst potential of all significant ionic species present in the external and internal solutions¹¹. The observation under these conditions of a blue light-stimulated outward current exceeding the zero current level (Fig. 3*b*) cannot be explained by passive ion fluxes, providing strong evidence for the operation of an electrogenic pump.

In the responses shown in Fig. 3, blue light induced outward currents as large as 5.5 pA. Given an average guard cell protoplast diameter of $14.1 \mu m$ ($n = 20$), currents as large as $0.88 \mu A cm^{-2}$ can be calculated. Previous observations of light-stimulated release of H^+ from guard cells^{4,5} suggest an electrogenic proton pump as the source of this current. Such a conclusion is further supported by the recent finding²⁰ that when suspensions of *V. faba* guard cell protoplasts illuminated with continuous high-intensity red light are exposed to a 30-s blue light pulse of the same fluence rate as that used in Figs 2 and 3, acidification of the medium occurs at rates comparable to the outward currents reported here.

In many types of plant cells²¹⁻²³, as well as in guard cells, H^+ extrusion appears to be coupled with K^+ uptake. The basis of that coupling is, however, unknown, available data being consistent with either an electrogenic H^+ pump or an antiporter with a stoichiometry for H^+ and K^+ which is not 1:1 (refs 24, 25). Our results showing electrogenic pumping in the absence of K^+ (Figs 2*b*, 3) clearly indicate that the electrogenic pump in guard cells which is activated by blue light is not an obligate K^+ antiporter. We conclude that the most likely mechanism underlying blue light stimulation of stomatal conductance to water vapour in the leaf (Fig. 1) is light activation of a hyperpolarizing proton pump in the guard cell plasmalemma which generates an electrical gradient for passive K^+ flux¹² driving increases in turgor. The data reported here therefore support a chemiosmotic model of stomatal opening¹³.

The results also have specific photobiological implications. The time course of the membrane hyperpolarization, particularly the delay between illumination onset and the initiation of hyperpolarization (Figs 2, 3), and the dependence of the response on ATP, are inconsistent with utilization of blue light as a source of photochemical energy for H^+ extrusion as occurs, for example, in *Halobacterium*²⁶. The response is observed when

guard cells are provided via the pipette solution with 9.2 mM ATP, a greater concentration than is typically measured in plant cells²⁷. Thus, it also seems unlikely that removal of an energy limitation on H^+ extrusion via blue light-induced enhancement of respiration²⁸ is occurring. Rather, blue light seems to function as a regulator of the electrogenic pump. Regulation could occur by a direct modulation of the membrane ATPase or by stimulation of redox reactions²⁹ that result in pump activation. Further application of the whole-cell patch-clamp technique to plant cells should prove valuable in elucidating the processes by which blue light and other environmental stimuli are transduced into cellular responses appropriate for plant function.

We thank Drs Eduardo Zeiger and Erwin Neher for use of equipment, comments on the manuscript and coordination of S.M.A.'s visit to the Department of Membrane Biophysics at the Max-Planck-Institut in Göttingen, where the patch-clamp experiments were performed. S.M.A. thanks also the members of the 1983 Cold Spring Harbor Single Channel Recording Course. This research was supported by a McKnight Fellowship to S.M.A. and Max-Planck stipendia to L.S. and J.I.S.

Received 24 June; accepted 2 October 1985.

- Spanwick, R. M. A. *Rev. Pl. Physiol.* **32**, 267-289 (1981).
- Schmidt, W. *Bioscience* **34**, 698-704 (1984).
- Zeiger, E. in *Blue Light Effects in Biological Systems* (ed. Senger, H.) 484-494 (Springer, Berlin, 1984).
- Raschke, K. & Humble, G. D. *Planta* **115**, 47-57 (1973).
- Gepstein, S., Jacobs, M. & Taiz, L. *Pl. sci. Lett.* **28**, 63-72 (1982).
- Zeiger, E. A. *Rev. Pl. Physiol.* **34**, 441-475 (1983).
- Sharkey, T. D. & Raschke, K. *Pl. Physiol.* **68**, 1170-1174 (1981).
- Ogawa, T., Ishikawa, H., Shimada, K. & Shibata, K. *Planta* **142**, 61-65 (1978).
- Hsiao, T. C., Allaway, W. G. & Evans, L. T. *Pl. Physiol.* **51**, 82-88 (1973).
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
- Marty, A. & Neher, E. in *Single Channel Recording* (eds Sakmann, B. & Neher, E.) 107-122 (Plenum, New York, 1983).
- Schroeder, J. I., Hedrich, R. & Fernandez, J. M. *Nature* **312**, 361-362 (1984).
- Zeiger, E., Bloom, A. J. & Hepler, P. K. *What's New Pl. Physiol.* **9**, 29-32 (1978).
- Iino, M., Ogawa, T. & Zeiger, E. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Schwartz, A. & Zeiger, E. *Planta* **161**, 129-136 (1984).
- Zeiger, E., Moody, W., Hepler, P. K. & Varela, F. *Nature* **270**, 270-271 (1977).
- Ishikawa, H., Aizawa, H., Kishira, H., Ogawa, T. & Sakata, M. *Pl. Cell Physiol., Tokyo* **24**, 769-772 (1983).
- Goldsmith, T. H. & Goldsmith, M. H. *Planta* **143**, 267-274 (1980).
- Sakmann, B. & Neher, E. (eds) in *Single-Channel Recording*, 37-51 (Plenum, New York, 1983).
- Shimazaki, K., Iino, M. & Zeiger, E. *Pl. Physiol.* **77S**, 164 (1985).
- Pitman, M. G., Schaefer, N. & Wildes, R. A. *Planta* **126**, 61-73 (1975).
- Lado, P., Rasi-Caldogno, F., Colombo, R., De Michelis, M. I. & Marre, E. *Pl. Sci. Lett.* **7**, 199-209 (1976).
- Beffagna, N., Cocucci, S. & Marre, E. *Pl. Sci. Lett.* **8**, 91-98 (1977).
- Poole, R. J. A. *Rev. Pl. Physiol.* **29**, 437-460 (1978).
- Bellando, et al. *Pl. Cell Envir.* **2**, 39-47 (1979).
- Lanyi, J. K. *Microbiol. Rev.* **42**, 682-706 (1978).
- Hampp, R., Goller, M. & Ziegler, H. *Pl. Physiol.* **69**, 448-455 (1982).
- Kowalik, W. A. *Rev. Pl. Physiol.* **33**, 51-72 (1982).
- Strasser, R. J. & Butler, W. L. in *The Blue Light Syndrome* (ed. Senger, H.) 205-211 (Springer, Berlin, 1980).
- Field, C., Berry, J. A. & Mooney, H. *Pl. Cell Envir.* **5**, 179-186 (1982).
- Gotow, K., Shimazaki, K., Kondo, N. & Syōno, K. *Pl. Cell Physiol.* **25**, 671-675 (1984).

Protection of cottontop tamarins against Epstein-Barr virus-induced malignant lymphoma by a prototype subunit vaccine

M. A. Epstein, A. J. Morgan, S. Finerty, B. J. Randle & J. K. Kirkwood

Department of Pathology, University of Bristol Medical School, University Walk, Bristol BS8 1TD, UK

Epstein-Barr (EB) virus is one of the five herpesviruses of man. Strong links between this agent and the chain of events causing two human cancers, endemic Burkitt's lymphoma and undifferentiated nasopharyngeal carcinoma, have long been evident (reviewed in ref. 1). Because of this, and because of the very high incidence of nasopharyngeal carcinoma in certain large populations², it was suggested in 1976 that a vaccine should be developed against EB virus to prevent infection and thereby reduce tumour incidence amongst those at risk³. The virus-determined membrane antigen

(MA) was proposed as immunogen³ because it was known to elicit naturally occurring virus-neutralizing antibodies in man⁴⁻⁷ and because analogous antigens had been shown to act as effective experimental vaccines for preventing the herpesvirus-induced lymphomas of Marek's disease in chickens^{8,9}. Progress has been achieved in defining, quantifying and preparing MA molecules, and in enhancing their immunogenicity¹⁰; a sensitive assay for antibodies to MA has been elaborated¹¹. Here we report that isolated cell membranes expressing MA, or purified MA glycoprotein of relative molecular mass (M_r) 340,000 (gp340), have been used to vaccinate cottontop tamarins (*Saguinus oedipus oedipus*), and that animals receiving either preparation were protected against the effects of a 100% tumour-inducing challenge dose of EB virus.

The cottontop tamarin is the only species in which experimental EB virus infection regularly causes lesions¹² and recent investigations have established that these are true, clonally derived, malignant lymphomas¹³. Young adult tamarins from a successful breeding colony¹⁴⁻¹⁶ were used in the present studies.

For the membrane experiments, B95-9 cells grown as reported earlier¹⁷ and showing maximum expression of MA after treatment with tetradecanoyl phorbol acetate¹⁸, were disrupted and the plasma membranes collected by sucrose density gradient centrifugation¹⁹. The resulting material contained only trace amounts of infectious virus when titrated in fetal cord blood lymphocytes²⁰, several logs less than is necessary for infection. The purified membranes were injected intraperitoneally (i.p.) eight times into two tamarins at fortnightly intervals, each inoculation containing on average 2,500 units of MA gp340 as assessed by radioimmunoassay (RIA)¹⁷. Serological responses were measured by indirect immunofluorescence, a highly sensitive enzyme-linked immunosorbent assay (ELISA)¹¹, and standard virus neutralization tests which assess the ability of a given serum to prevent transformation by the virus of fetal cord blood lymphocytes^{7,20}; all neutralizations were repeated (up to three times) and included control sera of known titre, so as to take account of the inherent variations in sensitivity shown by cells from different cord bloods. Specific antibodies were rapidly

Table 1 EB virus-induced lymphomas in unprotected cottontop tamarins

Expt no.	Lymphocyte-transforming units of virus injected	Injection route	Animals with gross multiple lesions
1	10 ^{5.3}	i.v.; i.p.; i.m.	2/2
2	10 ^{5.3}	i.p.; i.m.	2/2
3	10 ^{5.3}	i.p.; i.m.	4/4
4	10 ^{5.3}	i.p.; i.m.	2/2
5	10 ^{5.3}	i.p.; i.m.	4/4
6	10 ^{5.3}	i.p.; i.m.	2/2
7	10 ^{5.3}	i.p.; i.m.	2/2
8	10 ^{5.3}	i.p.; i.m.	2/2
9	10 ^{5.3}	i.p.; i.m.	4/4

induced, eventually reached high titre, and were then powerfully virus-neutralizing; because the neutralization tests were all repeated more than once, neutralizing titres were recorded as being greater than the minimum obtained in each set of tests. The tamarins were considered to have been satisfactorily immunized when their levels of neutralizing antibody equalled or exceeded the highest titres found in sera from naturally infected human subjects.

The immunized animals were challenged with 10^{5.3} lymphocyte-transforming units of virus²⁰, a dose checked in advance for its ability to produce gross, multiple, malignant tumours within 21 days¹³ in 100% of unprotected tamarins (Table 1). The tumours presented as masses (not infiltrates) in spleen, liver, kidneys, gut wall and adrenals, and in numerous lymph nodes, causing enlargement from the normal 1-2-mm diameter up to 15-20 mm (ref. 13). Use of a 100% lymphoma-inducing challenge dose of virus was considered important for biological tests in a species where only small numbers of animals are available and inbred strains are unknown, but to ensure exclusion of equivocal results, two additional normal control

Table 2 Protection of cottontop tamarins against EB virus-induced lymphomas by vaccination with B95-8 cell membranes

Tamarin	Antibody to B95-8 cell surface (immunofluorescence)	ELISA antibody titre	Virus neutralizing antibody	Lesions after 10 ^{5.3} units of virus i.p.; i.m.
	+++	1:800	Strong*	None
	+++	1:2,000	Strong*	None
	-	0	None	Gross multiple
	-	0	None	Gross multiple

* 1 ml serum neutralized >100,000 lymphocyte-transforming units of virus (from lowest value in repeated tests).

Table 3 Protection of cottontop tamarins against EB virus-induced lymphoma by vaccination with MA gp340 purified from B95-8 cell membranes by M_r -based method²¹

Expt	Tamarin	Antibody to MA (immunofluorescence)	ELISA antibody titre	Virus neutralizing antibody	Lesions after 10 ^{5.3} units of virus i.p.; i.m.
a, Pilot	Vaccinated	+++	1:1,000	Strong†	None
	Vaccinated	+	1:100	None	Gross multiple
	Vaccinated	+	<1:10	Slight	Gross multiple
	Vaccinated	+	*	None	Gross multiple
b, Confirmatory	Vaccinated	+++	1:800	Strong‡	2 Small, transient
	Vaccinated	+++	1:1,000	Strong§	Single, transient
	Control	-	0	None	Gross multiple
	Control	-	0	None	Gross multiple

* Serum sample haemolysed; not testable.

† 1 ml serum neutralized >10,000 lymphocyte-transforming units of virus.

‡ 1 ml serum neutralized 100,000 lymphocyte-transforming units of virus.

§ 1 ml serum neutralized >100,000 lymphocyte-transforming units of virus. All results are from the lowest value in repeated tests.

animals were also given the challenge material at the same time. The immunized tamarins remained entirely free of clinically detectable lesions whereas the normal control animals developed the expected gross multiple tumours within 2–3 weeks (Table 2).

Because it was known that purified MA gp340 prepared by a M_r -based method which conserved immunogenicity²¹ induced virus-neutralizing antibodies in various species when injected after incorporation in liposomes^{22,23}, we investigated the protective effects of these antibodies in tamarins, and the levels required for protection. In a pilot experiment, four animals were selected in whom differing amounts of neutralizing antibody were present after i.p. immunization with liposomal gp340, and all were then challenged with the 100% lymphomagenic dose of virus (Table 1). Table 3a shows that only the animal whose serum was strongly neutralizing was completely protected, whereas the other animals with much lower, or undetectable, levels of neutralizing antibody were not protected at all.

To confirm this result, two further tamarins were given gp340 in liposomes 17 times i.p. at fortnightly intervals, each dose containing on average 2,250 RIA units¹⁷. Antibodies were monitored as before and when these had reached high levels (Table 3b) the vaccinated animals were challenged with the 100% pathogenic dose of EB virus (Table 1), along with 2 normal control animals. The unprotected animals developed gross multiple tumours within 2–3 weeks whilst the vaccinated tamarins remained free of tumours (Table 3b); one protected animal exhibited minor transient swelling of a single inguinal lymph node draining the site of intramuscular (i.m.) injection in the thigh muscle, and the other developed similar inguinal node enlargement to 5 mm which rapidly regressed, accompanied by minor transient enlargement of a single mesenteric node.

These experiments demonstrate that gp340 prepared by the M_r -based method²¹ can be used as an efficient prototype subunit vaccine to protect susceptible experimental animals against tumour induction by a massive challenge dose of EB virus. The protected tamarins have remained well for more than 9 months and quite recent experiments with larger doses of gp340 have shown rapid induction of high-titre antibodies and followed the kinetics of their development²⁴. Investigation of the overall structure of the gp340 molecule has already indicated that more than 50% of its total mass is carbohydrate and that it is the protein component which is antigenic²⁵. The region of EB virus DNA carrying the gene for MA has been identified²⁶ and the nucleotide sequence of this gene is known²⁷; MA production by expression in pro- and eukaryotic cells therefore seems possible, and synthetic gp340 peptides could also be considered as immunogens, perhaps in combination with powerful new adjuvants²⁸. It should similarly be feasible to incorporate the MA gene into the genome of vaccinia virus to permit direct expression during vaccination with this agent^{29–31}. The present results establish a strong case for determined work in these directions to produce MA gp340 in a form suitable for use as a vaccine in humans.

This work was supported by the MRC (SPG978/32 and SPG780032) and the Cancer Research Campaign, London (out of funds donated by the Bradbury Investment Company of Hong Kong).

Received 13 May; accepted 6 September 1985.

- Epstein, M. A. & Achong, B. G. (eds) *The Epstein-Barr Virus* (Springer, Berlin, 1979).
- Shanmugaratnam, K. *Int. Rev. exp. Path.* **10**, 361–413 (1971).
- Epstein, M. A. *J. natn. Cancer Inst.* **56**, 697–700 (1976).
- Pearson, G., Dewey, F., Klein, G., Henle, G. & Henle, W. *J. natn. Cancer Inst.* **45**, 989–995 (1970).
- Pearson, G., Henle, G. & Henle, W. *J. natn. Cancer Inst.* **46**, 1243–1250 (1971).
- Gergely, L., Klein, G. & Ernberg, I. *Virology* **45**, 10–21 (1971).
- De Schryver, A. et al. *Int. J. Cancer* **13**, 353–362 (1974).
- Kaaden, O. R. & Dietzschold, B. *J. gen. Virol.* **25**, 1–10 (1974).
- Lesnick, F. & Ross, L. J. *N. Engl. J. Cancer* **16**, 153–163 (1975).
- Epstein, M. A. *Proc. R. Soc. B221*, 1–20 (1984).
- Randle, B. J. & Epstein, M. A. *J. virol. Meth.* **9**, 201–208 (1984).
- Miller, G. in *The Epstein-Barr Virus* (eds Epstein, M. A. & Achong, B. G.) 351–372 (Springer, 1979).
- Cleary, M. L. et al. *Science* **228**, 722–724 (1985).
- Kirkwood, J. K., Epstein, M. A. & Terlecki, A. J. *Lab. Anim.* **17**, 35–41 (1983).

- Kirkwood, J. K. *Primates* **24**, 515–520 (1983).
- Kirkwood, J. K., Epstein, M. A., Terlecki, A. J. & Underwood, S. J. *Lab. Anim.* (in the press).
- North, J. R., Morgan, A. J., Thompson, J. L. & Epstein, M. A. *J. virol. Meth.* **5**, 55–65 (1982).
- Hudewentz, J., Bornkamm, G. W. & zur Hausen, H. *Virology* **100**, 175–178 (1980).
- Crompton, M. J. & Snary, D. *Contemp. Topics molec. Immun.* **3**, 27–56 (1974).
- Moss, D. J. & Pope, J. H. *J. gen. Virol.* **17**, 233–236 (1972).
- Morgan, A. J., North, J. R. & Epstein, M. A. *J. gen. Virol.* **64**, 455–460 (1983).
- North, J. R., Morgan, A. J., Thompson, J. L. & Epstein, M. A. *Proc. natn. Acad. Sci.* **79**, 7504–7508 (1982).
- Morgan, A. J., Epstein, M. A. & North, J. R. *J. med. Virol.* **13**, 281–292 (1984).
- Epstein, M. A., Randle, B. J., Finerty, S. & Kirkwood, J. K. *Clin. exp. Immun.* (in the press).
- Morgan, A. J., Smith, A. R., Barker, R. N. & Epstein, M. A. *J. gen. Virol.* **65**, 397–404 (1984).
- Hummel, M., Thorley-Lawson, D. A. & Kieff, E. *J. Virol.* **49**, 413–417 (1984).
- Biggin, M., Farrell, P. J. & Barrell, B. G. *EMBO J.* **3**, 1083–1090 (1984).
- Morein, B., Sundquist, B., Höglund, S., Dalsgaard, K. & Osterhaus, A. *Nature* **308**, 457–460 (1984).
- Smith, G. L., Mackett, M. & Moss, B. *Nature* **302**, 490–495 (1983).
- Moss, B., Smith, G. L., Gerin, J. L. & Purcell, R. H. *Nature* **311**, 67–69 (1984).
- Mackett, M., Yilma, T., Rose, J. K. & Moss, B. *Science* **227**, 433–435 (1985).

Specific release of proteoglycans from human natural killer cells during target lysis

Reinhold E. Schmidt*, Richard P. MacDermott†‡, Gail Bartley*, Michael Bertovich†, David A. Amato‡, K. Frank Austen†, Stuart F. Schlossman*, Richard L. Stevens† & Jerome Ritz*

Divisions of *Tumor Immunology and ‡Biostatistics, Dana-Farber Cancer Institute, †Department of Rheumatology and Immunology, Brigham and Women's Hospital, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA

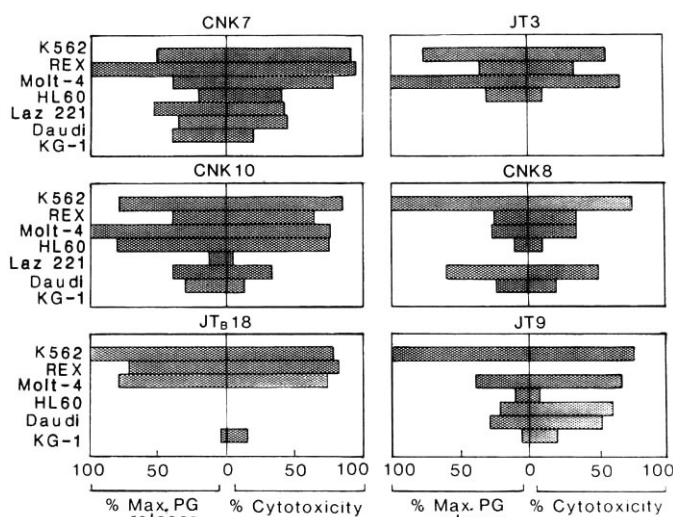
Natural killer (NK) activity is mediated by a small population of peripheral blood cells that exhibit the homogeneous morphology of large granular lymphocytes (LGL)^{1–4}. In recent studies, human NK cell clones^{5,6} have been shown to contain a 200,000- M_r (relative molecular mass) protease-resistant chondroitin sulphate A proteoglycan, which has been localized to the secretory granule by X-ray dispersive analysis and by its resistance to cleavage by extracellular addition of chondroitinase AC or ABC (ref. 7). In the present study, we have used six different human NK cell clones to demonstrate that release of ³⁵S-proteoglycan correlates closely with cytolytic activity against various NK cell targets. When NK activity is blocked by monoclonal antibodies at either the effector cell level (LFA-1)⁸ or at the target cell level (TNK_{TAR})⁹, there is a concomitant decrease in exocytosis of proteoglycan. Monoclonal antibodies directed against recognition structures, for example anti-NKTA and anti-T3 (ref. 10), function as soluble stimuli, capable of initiating the release of ³⁵S-proteoglycan. Taken together, these results provide strong evidence for the stimulus-specific release of chondroitin sulphate A proteoglycans from NK cells when the cytolytic process is activated.

To determine whether chondroitin sulphate A proteoglycan was consistently released in the cytolytic mechanism of NK cells, we used six different human NK cell clones selected because of their ability to maintain stable cytolytic activity against a series of allogeneic target cells without previous immunization^{5,11}. Phenotypic analysis of these clones indicated that each expressed NKH1 antigen, a surface structure of 200,000- M_r associated with all NK cells in peripheral blood¹², and the T11/E rosette receptor. Clones JT3, CNK7 and CNK10 also expressed NKH2 antigen¹², while clone JT_B18 was NKH2 negative. Together, these four clones, which do not express the T3 antigen or T-cell receptor-like structures, represent the most common phenotypes of circulating NK cells^{2–4,12}. Clones JT9 and CNK8 bear NKTA, a T-cell receptor-like structure that is linked to surface T3 (ref. 10). Moreover, both clones are specific

§ Present address: Division of Gastroenterology, Department of Medicine, Washington University, Barnes Hospital, St Louis, Missouri 63110, USA.

Fig. 1 Comparison of natural cytotoxicity and proteoglycan release from NK clones (CNK7, JT3, CNK10, CNK8, JT_B18 and JT9) interacting with seven target cell lines (K562, REX, Molt-4, HL60, Laz 221, Daudi and KG-1).

Methods. Cytotoxicity experiments were performed in triplicate using V-bottomed microtitre plates. Medium for cytotoxicity assays was RPMI 1640 containing 5% pooled human AB serum and 1% penicillin-streptomycin. Assays were performed at an effector/target cell ratio of 10:1 using 5,000 ⁵¹Cr labelled target cells per well. Cytotoxicity was determined after 4 h of incubation at 37 °C by measuring release of ⁵¹Cr into the cell supernatant. Specific cytotoxicity was calculated according to a standard method¹¹. Simultaneously, duplicate cultures of the same cloned NK cells were used in proteoglycan release experiments. For the latter studies, NK cells were incubated with 100 µCi of ³⁵S-sulphate ml⁻¹ at concentrations of 2 × 10⁶ cells ml⁻¹ for 24 h at 37 °C. After ³⁵S-sulphate labelling, the cloned NK cells were centrifuged and washed twice to remove unincorporated ³⁵S-sulphate. ³⁵S-labelled NK cells (25,000) were incubated with 50,000 target cells (effector/target ratio of 0.5:1) in quadruplicate in V-bottomed microtitre plates for 4 h at 37 °C. Supernatants (150 µl) from two of the four wells were combined, resulting in duplicate 300-µl samples for analyses. Each 300-µl supernatant sample was applied, together with 25 µl of chondroitin sulphate carrier and 250 µl of 8 M guanidin hydrochloride (GnHCl), to separate PD-10 columns (1.5 × 5.5 cm) which were equilibrated and eluted under dissociative conditions of 4 M GnHCl, 0.1 M Tris-HCl, 0.1 M Na₂SO₄, pH 7.0. Eight 0.5-ml fractions were collected and after the addition of 0.5 ml ethanol and 12.5 ml Hydrofluor to each fraction, ³⁵S-radioactivity was quantitated using a β-scintillation counter. The total proteoglycan content (TPG) was determined by addition of 1% Nonidet p-40. Spontaneous release (SR) was obtained from labelled effector cells incubated with media in the absence of targets (8.5 ± 4.5%, mean ± s.d.). Stimulus-specific per cent proteoglycan (PG) release was calculated according to the following formula, in which experimental release (ER) is release occurring in the presence of a target cell: % specific PG release = (ER - SR/TPG - SR) × 100. To compare the proteoglycan release of various NK clones in experiments done on different days, we expressed the release of labelled proteoglycan as a percentage of the maximal proteoglycan release obtained with the optimal NK/target combination in a given experiment which included K562 three times, Molt-4 twice, and REX once. In the experiments shown, maximal release represented 46% of total proteoglycan content for the combination CNK7/REX, 36% for CNK10/Molt-4, 48% for JT_B18/K562, 12% for JT3/Molt-4, 52% for CNK8/K562 and 69% for JT9/K562.



for a 140,000-*M_r* surface antigen that is linked to cell activation and is identified by two monoclonal antibodies, anti-TNK_{TAR} (ref. 9) and 4F2 (refs 13, 14), which have been shown to block target cell killing by NK clones expressing the NKTa clonotype.

Cytotoxicity was determined by measuring ⁵¹Cr release into the cell supernatant after incubation of unlabelled effector cells with ⁵¹Cr-labelled target cells at a ratio of 10:1 at 37 °C for 4 h¹¹. Release of ³⁵S-proteoglycan into the cell supernatant was determined during incubation of ³⁵S-labelled effector cells with unlabelled target cells at a ratio of 0.5:1 for 4 h at 37 °C. To compare proteoglycan release by different targets with toxicity against these cells, the maximum release of ³⁵S-proteoglycan from each NK clone in a given experiment was considered to represent 100% release and all other release values were expressed as a per cent of maximal release. As shown in Fig. 1, specific cytotoxicity closely paralleled proteoglycan release; this was found for T3-positive (T3⁺) as well as for T3-negative (T3⁻) NK clones. Figure 2 summarizes the results of 34 experiments using six different NK clones and seven different targets; there was a linear relationship between percentage cytotoxicity and percentage maximal proteoglycan release (correlation coefficient 0.81).

The specific exocytosis of ³⁵S-proteoglycan was also evaluated in conditions in which NK cytotoxicity was inhibited at either the effector cell or target cell level. Monoclonal antibody 2F12, which is specific for LFA-1 antigen^{15,16} and is known to block cytotoxicity of NK clones at the effector cell level⁸, inhibited cytotoxicity of both T3⁺ and T3⁻ NK clones, with the greatest inhibition occurring with Molt-4, CEM and REX targets (Fig. 3). Reductions in exocytosis of ³⁵S-proteoglycans closely paralleled the inhibition of cytotoxicity. The monoclonal antibody anti-TNK_{TAR} inhibited the NK cytotoxicity at the target cell level of the T3⁺ clones JT9 and CNK8, both of which express the NKTa clonotype (Fig. 3). In these experiments, specific release of proteoglycans from JT9 and CNK8 was also markedly reduced, whereas there was no change in either cytolytic function or release of proteoglycans from clones JT_B18 and JT3. The correlation coefficient between percentage maximal

proteoglycan release and percentage cytotoxicity for all experiments shown in Fig. 3 was calculated to be 0.92.

Release of proteoglycans from NK cell clones was also evaluated under conditions known to trigger activation of NK cells without the presence of target cells. The results (Table 1) demonstrate that binding of monoclonal anti-T3 and anti-NKTa antibodies was effective in triggering release of ³⁵S-proteoglycans from NK cell clones JT9 and CNK8 but not from JT_B18 and JT3. The former two clones, but not the latter two, are known to use a T-cell receptor-like complex in expressing their cytolytic function¹⁷. Monoclonal anti-T6, which was used as a negative control antibody, did not initiate any exocytosis of proteoglycan. In contrast, the lectins concanavalin A (Con A) and Phytohaemagglutinin (PHA) were able to trigger release of proteoglycan from all NK clones regardless of differences in cell surface phenotype, with PHA being more effective than Con A. Control experiments using these monoclonal antibodies showed that the viability of the cells before and after triggering by trypan blue exclusion was never less than 95% and that the lactate dehydrogenase release into the cell supernatant was never greater than 5%.

The data presented here provide strong evidence that proteoglycan exocytosis occurs concomitantly with stimulus-specific

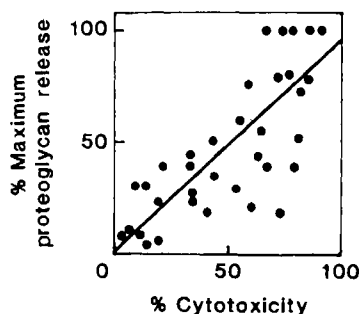


Fig. 2 Correlation of per cent specific cytotoxicity with per cent maximal proteoglycan release for 34 different experiments. $r = 0.81$.

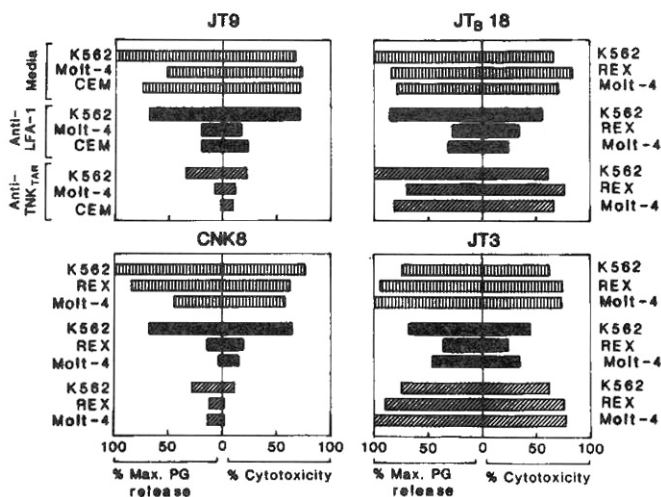


Fig. 3 Natural cytotoxicity and proteoglycan release are inhibited by anti-LFA-1 and anti-TNK_{TAR} antibodies, which exert their blocking activity through epitopes on the NK and target cells, respectively. Either effector cells or target cells were incubated with media (□) or monoclonal antibody anti-LFA-1 (▨) or anti-TNK_{TAR} (■) for 30 min at room temperature before the assay, and both specific cytotoxicity and per cent maximal proteoglycan release were measured as described in Fig. 1 legend, except that cytotoxicity experiments were performed using an effector/target cell ratio of 5:1. Maximal proteoglycan release was 65% of total proteoglycan content for the combination JT9/K562, 46% for CNK8/K562, 46% for JT18/K562 and 13% for JT3/Molt-4.

activation of the cytolytic capacity of NK cells. In a companion study of cloned NK cells, ³⁵S-proteoglycan was demonstrated to be localized in intracellular granules by X-ray dispersive analysis and was shown to be a protease-resistant chondroitin sulphate A proteoglycan⁷. By using a panel of six human NK cell clones and a variety of different target cells, we now also demonstrate a linear correlation between specific cytotoxicity and release of proteoglycan (Figs 1, 2), indicating that this secretory granule constituent is specifically released during the NK cytolytic process. This conclusion is further supported by the demonstration that blocking of NK activity by monoclonal antibodies anti-LFA-1 and anti-TNK_{TAR}, which inhibit cytotoxicity at the effector cell and target cell level, respectively, also concurrently inhibit the release of intracellular proteoglycans (Fig. 3). In addition, triggering of NK clones with soluble agonists through specific receptors or through binding of Con A and PHA similarly resulted in the release of radiolabelled proteoglycans (Table 1).

Protease-resistant chondroitin sulphate A proteoglycan may function as a carrier for cytolytic proteins or may protect the NK cell from injury by the cytotoxic molecules which are released during the lethal reaction^{18,19}. The results presented define the secretory nature of the NK cellular response in terms of a secretory granule marker, intracellular proteoglycan, and are compatible with the stimulus-specific nature of target cell cytotoxicity.

Table 1 ³⁵S-Proteoglycan release by NK clones activated with soluble stimuli

NK clone	Anti-T6	Anti-T3	Anti-NKTa	Con A	PHA
JT18	0	0	0	17	26
JT3	0	0	0	7	63
JT9	0	25	28	20	59
CNK8	0	46	40	14	29

Values are per cent total proteoglycan release from various cloned NK cells incubated for 4 h with monoclonal antibodies anti-T6 (control), anti-T3 and anti-NKTa, or the lectins PHA (20 µg ml⁻¹) and Con A (20 µg ml⁻¹). Proteoglycan release was determined as described in Fig. 1 legend.

This work was supported in part by NIH grants AI-22531, M-21474, AM-35984, CA-34183, CA-06516 and HL-17382. R.E.S. is the recipient of a fellowship (Schm 596/1-1) from the Deutsche Forschungsgemeinschaft. R.P.MacD. is a recipient of National Research Service award F33 AM-07463. J.R. is a Scholar of the Leukemia Society of America.

Received 23 July; accepted 24 September 1985.

- Herberman, R. B. & Ortaldo, J. R. *Science* **214**, 24-30 (1981).
- Trinchieri, G. & Perussia, B. *Lab. Invest.* **50**, 489-513 (1984).
- Ortaldo, J. R., Sharrow, S. H., Timonen, T. & Herberman, R. B. *J. Immun.* **127**, 2401-2409 (1981).
- Zarling, J. M., Clouse, K. A., Biddison, W. E. & Kung, P. C. *J. Immun.* **127**, 2575-2580 (1981).
- Hercend, T., Reinherz, E. L., Meuer, S., Schlossman, S. F. & Ritz, J. *Nature* **301**, 158-159 (1983).
- Schmidt, R. E., Bartley, G., Levine, H., Schlossman, S. F. & Ritz, J. K. *J. Immun.* **135**, 672-678 (1985).
- MacDermott, R. P. *et al. J. exp. Med.* (in the press).
- Schmidt, R. E. *et al. J. Immun.* **135**, 1020-1025 (1985).
- Hercend, T. *et al. Eur. J. Immun.* **14**, 844-852 (1984).
- Hercend, T. *et al. J. exp. Med.* **158**, 1547-1560 (1983).
- Hercend, T., Meuer, S. C., Reinherz, E. L., Schlossman, S. F. & Ritz, J. *J. Immun.* **129**, 1299-1305 (1982).
- Hercend, T. *et al. J. Clin. Invest.* **75**, 932-943 (1985).
- Haynes, B. F. *et al. J. Immun.* **126**, 1409-1414 (1981).
- Moingeon, P. *et al. J. Immun.* **134**, 2930-2934 (1985).
- Hildreth, J. E. K., Gotch, F. M., Hildreth, P. D. K. & McMichael, A. J. *Eur. J. Immun.* **13**, 202-208 (1983).
- Springer, T. A. *et al. Immun. Rev.* **68**, 171-192 (1982).
- Ritz, J. *et al. Science* **228**, 1540-1543 (1985).
- Henkart, P. A., Millard, P. J., Reynolds, C. W. & Henkart, M. P. *J. exp. Med.* **160**, 75-93 (1984).
- Pasternak, M. S. & Eisen, H. N. *Nature* **314**, 743-745 (1985).

A trans-acting class II regulatory gene unlinked to the MHC controls expression of HLA class II genes

C. de Préval, B. Lisowska-Grospierre*, M. Loche, C. Griscelli* & B. Mach

Department of Microbiology, University of Geneva School of Medicine, CH-1211, Geneva 4, Switzerland, and Unité d'Immunologie Pédiatrique, INSERM U 132, Hôpital des Enfants Malades, Paris Cedex, France

Class II (or Ia) antigens are highly polymorphic surface molecules which are essential for the cellular interactions involved in the immune response. In man, these antigens are encoded by a complex multigene family which is located in the major histocompatibility complex (MHC) and which comprises up to 12 distinct α - and β -chain genes, coding for the HLA-DR, -DQ and -DP antigens^{1,2}. One form of congenital severe combined immunodeficiency (SCID) in man, which is generally lethal, is characterized by an absence of HLA-DR histocompatibility antigens on peripheral blood lymphocytes (HLA class II-deficient SCID). In these patients, as reported here, we have observed an absence of messenger RNA for the α - and β -chains of HLA-DR, -DQ and -DP, indicating a global defect in the expression of all class II genes. Moreover, the lack of expression of HLA class II mRNAs could not be corrected by γ -interferon, an inducer of class II gene expression in normal cells. Family studies have established that the genetic defect does not segregate with the MHC. We conclude, therefore, that the expression of the entire family of class II genes is normally controlled by a trans-acting class II regulatory gene which is unlinked to the MHC and which is affected in the patients. This gene controls a function or a product necessary for the action of γ -interferon on class II genes.

The expression of the entire class II multigene family is restricted to a limited number of cell types, is strictly regulated developmentally, and is coordinately induced by soluble mediators (such as γ -interferon). Cells from class II-deficient SCID cases could thus provide a model for the study of the regulation of this gene family. In these patients, the absence of expression of class II antigens on lymphocytes and macrophages³ explains the immunological unresponsiveness

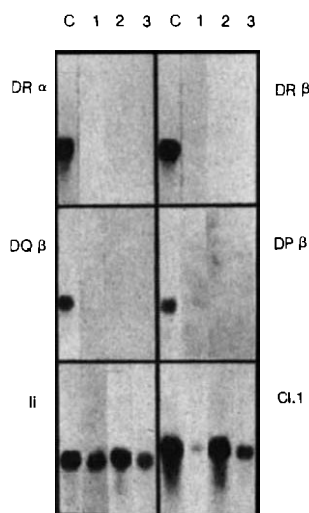


Fig. 1 Northern blot analysis of mRNAs from PHA-stimulated blasts of HLA class II-deficient SCID patients (lanes 1-3) and of a normal healthy donor (C).

Methods. RNA preparation and Northern blot analysis were performed as reported elsewhere²⁰. Briefly, total RNA from PHA-blasts was prepared by the guanidinium thiocyanate-caesium chloride procedure, denatured in glyoxal and electrophoresed in a 1.8% agarose gel (20 g per slot). RNA was transferred to diazobenzylmethyl paper, and the filters were hybridized in 50% formamide, 0.75 M NaCl, 10% dextran sulphate at 42 °C, with nick-translated ³²P-cDNA probes (specific activity 1.5–3 × 10⁸ c.p.m. g⁻¹). The final washes were done under stringent conditions: 0.1 × SSC buffer, 68 °C, twice for 30 min each before autoradiography. The following ³²P-labelled probes were used: DRα⁵, DRβ and DQβ⁶, DPβ⁷, invariant chain (Ii)⁸, and class I heavy-chain pHLA 44/2 (CI.1; ref. 21). Some blots were dehybridized in 99% formamide, 5 mM Tris-HCl pH 7.5, 1 mM EDTA, 65 °C, three times for 10 min each, and rehybridized. The specific probe used for each of the six experiments is indicated. The films were exposed for 4 days; prolonged exposure (17 days) gave identical results (data not shown). RNAs from fresh peripheral blood lymphocytes from one patient gave identical results.

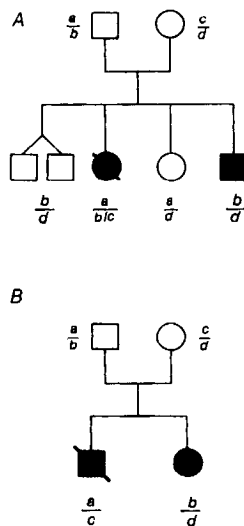
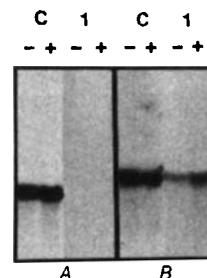


Fig. 2 Segregation of HLA haplotypes in two families. Open symbols, healthy individuals; solid symbols, HLA class II-deficient SCID patient; ●, ■, deceased individuals. A, The healthy twins and their affected brother are haplo-identical with respect to HLA. Haplotypes: a = A2 B12; b = Aw32 B40; c = A1 B21; d = Aw32 B8. B, The two immunodeficient siblings do not have any HLA haplotypes in common. Haplotypes: a = A26 B35 DR2; b = A19.2 B35 DR3; c = A19.2 B7 DR1; d = A3 B7.

Fig. 3 Northern blot analysis of lymphocyte mRNA from HLA class II-deficient SCID patients (lanes labelled 1) and a healthy donor (C) following *in vitro* stimulation with γ -interferon. The probes used were: A, DRβ; B, CI.1.

Methods. Blood lymphocytes from patient 1 and from a healthy donor were cultured for 48 h in RPMI 1640 medium, 10% fetal calf serum, in the presence of recombinant interferon (500 U ml⁻¹), provided by Biogen SA. RNA preparation and Northern blot analysis were performed as described in Fig. 1 legend. The blot in A was first hybridized with DRβ cDNA⁶; the final washes were performed in 0.5 × SSC buffer at 65 °C. (In these conditions, the DRβ ³²P-labelled cDNA also hybridizes to DQβ and DPβ mRNAs.) This blot was dehybridized and rehybridized with an HLA class I ³²P-cDNA probe, CI.1 (ref. 20).



and the dramatic clinical course, which results frequently in death. In 12 patients with HLA class II-deficient SCID, we had observed an absence of HLA-DR molecules on peripheral blood lymphocytes, even after stimulation with phytohaemagglutinin (PHA)⁴. Other cells of the body that are normally HLA-DR-positive were also totally negative. In three of the patients, we have studied the expression of the different genes of the entire *HLA-D* region, using specific HLA class II α- and β-chain complementary DNA probes, described previously⁵⁻⁷.

Messenger RNA for HLA-DR, -DQ and -DP antigens (α- or β-chains) could be easily detected in blood lymphocytes from normal individuals. In contrast, no class II mRNA was found in samples of stimulated lymphocytes from patients with HLA class II-deficient SCID (Fig. 1), even after prolonged exposure of the autoradiographs. This block in mRNA expression concerns the three subregions *HLA-DR*, *-DQ* and *-DP*. However, a normal amount of mRNA for the HLA-DR-associated invariant chain (Ii)⁸ was observed. This difference is of interest in view of the general coupling of expression of the *Ii* gene with that of HLA class II genes both in specific tissues and following induction with γ -interferon^{9,10}. RNA for HLA class I antigens either was present in a normal amount or was somewhat decreased.

SCID seems to be inherited as an autosomal recessive trait, with a high rate of consanguinity and a high frequency of families with two or more affected children of both sexes^{3,4}. As the defect of class II expression concerns not only bone marrow-derived cells, but also all cells that can express class II antigens in normal individuals, we are dealing with a germline defect which consists of a global inability to express HLA class II genes in the form of mRNA. The defect is not only global in terms of all the cell types involved, but it is also multigenic and involves each of the multiple α- and β-chain genes within the *HLA-D* region. Individual mutations in each of these genes are unlikely and massive deletions have been ruled out as a possible explanation by the finding of a normal restriction pattern for *DR* and *DC* genes by Southern blot hybridization¹¹. Cells from one class II-deficient SCID patient were found to revert to a HLA-DR-positive phenotype after 3–5 weeks in Epstein-Barr virus-induced culture⁴. Another argument for the structural integrity of class II genes in these patients comes from an analysis of the family pedigree, including the HLA typing of the parents and relatives. Figure 2A shows a family in which two children are haplo-identical for the MHC, yet one is healthy and the other is affected. Figure 2B illustrates another family in which two siblings are non-identical with respect to the MHC, yet both are affected. Therefore, it can be concluded that the genetic defect which results in a block of class II gene expression does not segregate with the MHC. To our knowledge, no animal model with such a mutation has yet been described.

γ -Interferon is known to stimulate the expression of class II genes, even in cells that do not normally express them^{9,10}. However, the lack of expression of class II genes in HLA class II-deficient SCID could not be corrected by γ -interferon. Figure 3 shows the result of an analysis of RNA from lymphocytes

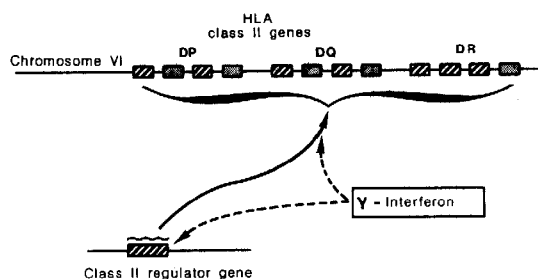


Fig. 4 A class II regulatory gene maps outside the major histocompatibility complex. This gene, or its product, is a potential target for γ -interferon (see text).

from a patient and from a normal control, following incubation in the presence and absence of γ -interferon. The unresponsiveness to interferon of the class II genes contrasts with the response of class I genes (Fig. 3), indicating that the cells from the immunodeficient individuals possess the γ -interferon receptor and are capable of responding.

In these SCID patients, therefore, the class II structural genes are intact and the defect in their expression is caused by a mutation(s) in a gene located outside the MHC. Thus, a *trans*-acting gene unlinked to the MHC is involved in the normal regulation of the expression of the class II multigene family (Fig. 4). The exact mechanism of this regulation could be transcriptional or post-transcriptional. In this regard, it is of interest that all class II genes of man and mouse (α - and β -genes) share two short sequences 5' upstream of the genes, referred to as 'class II boxes'^{12,13}. From the unresponsiveness of class II-deficient SCID cases to γ -interferon, it is also evident that the induction of class II gene expression observed with γ -interferon in normal cells requires the normal functioning of the class II regulatory gene. The target for the effect of γ -interferon could be the regulatory gene itself, or its product. The expression of the invariant chain gene, although induced by γ -interferon, is probably not under the control of the same regulatory gene.

It is possible that the DR-negative variants^{14,15} in which we had also observed an absence of class II mRNA with the presence of mRNA for class I and invariant chains^{16,17}, represent an analogous situation. Interestingly, fusion with a DR-positive B cell has resulted in the re-expression of the DR antigen of the variant type¹⁸. It has also been shown that fusion of a human DR-positive line with several mouse Ia-negative lines resulted in a DR-negative phenotype, even when the human MHC was still present in the hybrid cells¹⁹. The latter observation is also compatible with the existence of a class II regulatory gene, which would be lost in most of these fusions. Finally, the model presented in Fig. 4 can also accommodate a class II regulation of a negative kind (analogous to the repressors of prokaryotes) in which induction of class II genes would normally result from the inhibition of a negative signal.

The study of HLA class II-deficient patients has thus provided evidence for a novel gene controlling the expression of Ia antigens. One can anticipate that, in the future, this gene may be isolated and transfected into bone marrow stem cells of immunodeficient patients in order to achieve somatic correction of the disease.

This work was supported by the Swiss National Fund for Scientific Research, and by fellowships from EMBO and the Zyma Foundation.

Received 15 July; accepted 19 September 1985.

- Hood, L., Steinmetz, M. & Malissen, B. *A. Rev. Immun.* 1, 529-568 (1983).
- Mach, B. *Scand. J. Immun.* 20, 473-478 (1984).
- Grisicelli, C. *et al.* in *Progress in Immunodeficiency Research and Therapy* Vol. 1 (eds Grisicelli, C. & Voosen, J.) 19-26 (Excerpta Medica, Amsterdam, 1984).
- Lisowska-Groszperle, B. *et al. J. clin. Invest.* (in the press).
- Wake, C. T. *et al. Proc. natn. Acad. Sci. U.S.A.* 79, 6979-6983 (1982).
- Long, E. O. *et al. Proc. natn. Acad. Sci. U.S.A.* 79, 7465-7469 (1982).
- Goraki, J., Rollini, P., Long, E. O. & Mach, B. *Proc. natn. Acad. Sci. U.S.A.* 81, 3934-3938 (1984).

- Strubin, M., Mach, B. & Long, E. O. *EMBO J.* 3, 869-872 (1984).
- Collins, T. *et al. Proc. natn. Acad. Sci. U.S.A.* 81, 4917-4921 (1984).
- de Prével, C., Gorski, J. & Mach, B. in *Histocompatibility Testing 1984* (eds Albert, E. D., Baur, M. P. & Mayr, W. R.) 569-572 (Springer, Berlin, 1984).
- Marcadet, A. *et al. New Engl. J. Med.* 312, 1287-1292 (1985).
- Saito, H., Maki, R. A., Clayton, L. K. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* 80, 5520-5524 (1983).
- Kelly, A. & Trowsdale, J. *Nucleic Acids Res.* 13, 1907-1921 (1985).
- Gladstone, P. & Pious, D. *Nature* 271, 459-461 (1978).
- Accolla, R. S. *J. exp. Med.* 157, 1053-1058 (1983).
- Long, E. O., Mach, B. & Accolla, R. S. *Immunogenetics* 19, 349-353 (1984).
- Levine, F., Erlich, A. H., Mach, B. & Pious, D. *J. Immun.* 134, 637-640 (1985).
- Gladstone, P. & Pious, D. *Somatic Cell Genet.* 6, 285-298 (1980).
- Benham, F. J., Quintero, M. A. & Goodfellow, P. N. *EMBO J.* 2, 1963-1968 (1983).
- de Prével, C. & Mach, B. *Immunogenetics* 17, 133-140 (1983).
- Malissen, M., Damotte, D., Birnbaum, D., Trucy, J. & Jordan, B. *Gene* 20, 485-489 (1982).

A role for N_i in the hormonal stimulation of adenylate cyclase

Richard A. Cerione*, Claudia Staniszewski*, Marc G. Caron*, Robert J. Lefkowitz*, Juan Codina† & Lutz Birnbaumer†

* Howard Hughes Medical Institute, Departments of Medicine (Cardiology), Biochemistry and Physiology, Duke University Medical Center, Durham, North Carolina 27710, USA

† Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030, USA

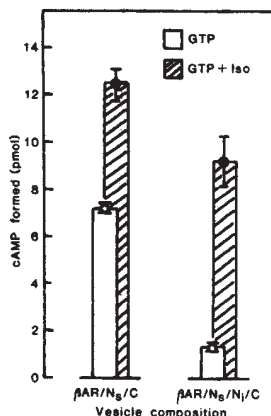
The best understood system for transduction of extracellular messages into intracellular signals is the hormone receptor-coupled adenylate cyclase. In such systems receptors are functionally coupled to the enzyme by two special proteins, termed the stimulatory and inhibitory guanine nucleotide regulatory proteins (N_s and N_i , respectively). These proteins, thought to mediate, respectively, stimulatory and inhibitory influences on the adenylate cyclase, are members of a larger class of heterotrimeric guanine nucleotide regulatory proteins involved in membrane signal transduction¹⁻⁵. We have studied the interactions of the various components of the adenylate cyclase system by co-reconstituting pure β -adrenergic receptors, pure N_s and N_i , and functionally resolved preparations of the catalyst in phospholipid vesicles. In the absence of N_i , β -adrenergic receptor/ N_s -mediated catecholamine stimulation of the enzyme is relatively modest (~1.3-fold). Surprisingly, however, when N_i is also present, stimulation increases dramatically (up to 7-8-fold) because of a greater suppression of basal relative to agonist-stimulated enzyme activity. Thus, N_i may actually be required for maximal agonist stimulation as well as for inhibition of the adenylate cyclase.

It is believed that hormonal stimulation of the adenylate cyclase (C) system is a simple vectorial signal transduction process whereby the receptor first facilitates the activation of N_s by GTP and the GTP/ N_s complex then activates adenylate cyclase. The stimulation process is terminated by the GTPase activity of N_s . Recently, we reported that the co-insertion into phospholipid vesicles of the pure guinea pig lung β -adrenergic receptor (β AR), with the pure human erythrocyte N_s protein and a resolved bovine caudate adenylate cyclase preparation (that is, resolved from β AR and N_s by Sepharose-6B chromatography) indeed results in the establishment of a modest responsiveness of adenylate cyclase activity to isoprenaline⁶. The results of Fig. 1 confirm this observation. For the conditions shown in Fig. 1 the maximal stimulation by isoprenaline never exceeds two fold and in some cases is nearly non-existent. The average (fold) stimulation by isoprenaline in 11 such experiments is 1.32 ± 0.07 (mean $\pm$ s.e.m.). It seems likely that the modest effect of isoprenaline in these three component systems reflects, at least in part, the effective activation of N_s molecules by GTP alone, thus resulting in a significant basal level of cyclic AMP production.

Fig. 1 The effect of N_i on β -adrenergic-mediated adenylate cyclase activity in phospholipid vesicles. The data are presented as the means of triplicate determinations, with the ranges denoted by error bars.

Methods. Resolved bovine caudate adenylate cyclase (C) preparations (150 μ l; see below), 1.14 μ g of human erythrocyte N_s (≈ 12 pmol), 12 pmol of guinea pig lung β -adrenergic receptor (β AR) and 1.8 μ g of human erythrocyte N_i (≈ 19 mol) were inserted into phospholipid vesicles as follows. Soybean phosphatidylcholine (0.1 ml of a 17 mg ml⁻¹ sonicated solution; Sigma) was incubated with the adenylate cyclase preparation (0.15 ml), 0.08–0.12 ml of 100 mM NaCl, 10 mM Tris-HCl pH 7.4 and 25 μ l of octyl glucoside (17%) for 20–30 min on ice. At this point the nucleotide regulatory proteins (30 μ l of N_s , 36 μ l of N_i) and the receptor [40 μ l of β AR which had been preincubated with 10 μ l of alprenolol (5 μ M) and 10 μ l of bovine serum albumin (BSA, 50 mg ml⁻¹)] were added to the lipid solutions, which had been incubated for ~1–2 min at 4 °C. Rhodopsin (195 pmol) was also included in the incubations containing N_i as we had found previously that rhodopsin promotes the maximal activation of this regulatory protein¹¹. However, under the conditions described for these experiments, N_i alone is effectively activated and essentially identical results are obtained in the absence of rhodopsin. These incubation mixtures were then immediately applied (at 4 °C) to Extracti-gel columns (1 ml of gel) pretreated with 4 vol. of 100 mM NaCl, 10 mM Tris-HCl pH 7.4 containing 2 mg ml⁻¹ of BSA, then equilibrated with 100 mM NaCl, 10 mM Tris-HCl pH 7.4. The protein-vesicles, in the column eluate, were then pelleted using polyethylene glycol essentially as described previously¹¹. The resultant protein-lipid pellets were resuspended in 75 mM Tris-HCl pH 7.8, 1 mM dithiothreitol (DTT, final volume 0.35 ml) and 20 μ l of the vesicles were assayed for adenylate cyclase activity as described below. The β -adrenergic receptor was purified ($\geq 95\%$) from guinea pig lung membranes as described previously¹⁶. The pure receptor preparations were concentrated to ~300 pmol ml⁻¹ using an Amicon concentration cell with a YM 30 membrane, and then stored at -90 °C in 50 mM Tris-HCl, 50 mM NaCl, 0.2% digitonin pH 7.4. N_s and N_i were purified ($>90\%$) as described by Codina *et al.*¹² and stored at -90 °C in 10 mM HEPES pH 8.0, 1 mM EDTA, 20 mM β -mercaptoethanol, 30% ethylene glycol, 150 mM NaCl, 50 μ g ml⁻¹ BSA and in 1.65–10% Lubrol PX. The catalytic moiety (C) of adenylate cyclase was solubilized from bovine caudate nucleus with sodium cholate and isolated from the other components of the system by Sepharose-6B chromatography as described elsewhere^{6,17}. These preparations were stored in 50 mM Tris-HCl, 200 mM sucrose, 1 mM DTT, 15 mM MgCl₂, 3.5 mg ml⁻¹ crude soybean phosphatidylcholine and 0.6% sodium cholate, pH 7.6. The concentrations of β AR are expressed in terms of ¹²⁵I-iodocyanopindolol binding activity. The amounts of N_s and N_i are expressed in terms of total protein as described previously¹²; the molar concentrations of these proteins were determined assuming a relative molecular mass of 95,000 (ref. 12). The adenylate cyclase assays were performed in a total volume of 0.1 ml containing 50 mM Na-HEPES pH 8.0, 1 mM EDTA, 0.25 mM ATP, 3 mM phosphoenolpyruvate, 10 μ g ml⁻¹ pyruvate kinase, 0.1 mg ml⁻¹ BSA, 0.1 mM cyclic AMP (cAMP), 10 μ g ml⁻¹ myokinase, 2 μ Ci of [α -³²P]ATP and 2 mM MgCl₂. Isoprenaline (Iso) = 0.1 mM; GTP = 10 μ M.

The construction of a vesicle system comprised of pure β AR, pure N_s , resolved adenylate cyclase and pure human erythrocyte N_i results in three striking effects (Fig. 1): (1) the absolute levels of adenylate cyclase activity are reduced (relative to β AR/ N_s /C vesicles) both when assayed in the absence and in the presence of isoprenaline; (2) the reduction of activity in the absence of isoprenaline is much more marked than in its presence; and due to this, (3) the relative stimulation of adenylate cyclase activity by isoprenaline ('fold stimulation') is increased. In the case shown in Fig. 1, the stimulation of adenylate cyclase activity by isoprenaline is 1.8-fold in the β AR/ N_s /C vesicles and increases



to 7-fold in the β AR/ N_s / N_i /C vesicles. In a series of 11 experiments the 'fold' stimulation of the enzyme by isoprenaline was 1.32 ± 0.07 in the absence and 3.61 ± 0.6 in the presence of N_i . We have documented that the effects of N_i in the reconstituted system were not the result of preferential reduction of the basal (GTP) activities due to a reduced incorporation of adenylate cyclase or N_s or of any change in the efficiency of β AR reconstitution (data not shown).

Figure 2a demonstrates clearly that the ability of N_i to increase the relative stimulation of adenylate cyclase activity by isoprenaline is a dose-dependent phenomenon. At the highest level of N_i incorporated, the adenylate cyclase activity assayed in the absence of isoprenaline was inhibited by ~85%, while activity measured in the presence of isoprenaline was inhibited by ~42% (Fig. 2b); this results in an increase in the stimulation of adenylate cyclase activity by isoprenaline from ~1.1-fold for control vesicles, to 4.1-fold in vesicles containing N_i . The mean inhibitions of basal and isoprenaline-stimulated activities by N_i were, respectively, $76 \pm 3\%$ and $43 \pm 4\%$ ($n = 11$). In a separate series of experiments using the resolved subunits of N_i or transducin, we have found that the activities of N_i described here are essentially properties of the $\beta\gamma$ - rather than the α -subunit (manuscript in preparation).

Our results indicate a dual role for the inhibitory nucleotide binding regulatory protein (N_i) of the adenylate cyclase system, one being the mediation of inhibition of adenylate cyclase activity, the other being a tightening of the coupling between adenylate cyclase activity and stimulatory hormones by the marked attenuation of the basal (N_s -mediated) adenylate cyclase activity.

Moreover, not only do our results suggest a dual role for N_i , they also imply two distinct functions for the stimulatory hormone/receptor complex. The first is to accelerate the actual activation of N_s . The second is to suppress the inhibitory action of N_i on GTP-activated N_s . Evidence for the first function derives from studies in which it has been shown that β -agonists promote the interaction between the pure β AR and N_s in phospholipid vesicles, leading to increases in the rate of GTP γ S binding to N_s and the turnover number for the GTPase activity^{7–11}. Evidence for the second function of the hormone/receptor complex is provided by the present work, which indicates that addition of agonists to vesicles containing β AR/ N_s / N_i /C markedly relieves the N_i inhibition, leading to a significant increase in hormonal stimulation.

Of further interest is the fact that purification studies have indicated that in native membranes there is 4–10 times more N_i than N_s (ref. 12), this agrees well with the relative ratios of N_i / N_s (4–5 times) required in our reconstitution systems to both inhibit GTP-activated N_s and enhance the relative stimulation by hormone.

The specific mechanisms by which N_s and N_i are activated under physiological conditions (that is, in the presence of stimulatory and inhibitory receptors, GTP and in a lipid milieu) still remain to be delineated. Both N_s and N_i are heterotrimeric in structure ($\alpha\beta\gamma$) and in detergent solution their activation by non-hydrolysable analogues of GTP involves subunit dissociation into α_s (ref. 13) or α_i (ref. 14), and an intact $\beta\gamma$ species J.C., J. D. Hildebrandt & L.B., unpublished results. It has been suggested that it is the α_s /guanine nucleotide complex which stimulates adenylate cyclase activity. However, the mechanism by which inhibition occurs has been a more controversial subject. It has been suggested that it is the generation of $\beta\gamma$ following N_i activation (and dissociation) which effectively complexes activated α_s (refs 1, 14), while other studies in N_s -deficient S49 *cyc*⁻ cells suggest a direct inhibitory action of α_i on adenylate cyclase¹⁵. Recent studies using phospholipid vesicle systems similar to those described here indicate that bovine brain N_i or bovine retinal transducin inhibit the N_s -stimulated adenylate cyclase rather than the intrinsic activity of the enzyme itself¹⁹. Moreover, experiments using the isolated subunits of these proteins indicate that the $\beta\gamma$ subunit complex is primarily

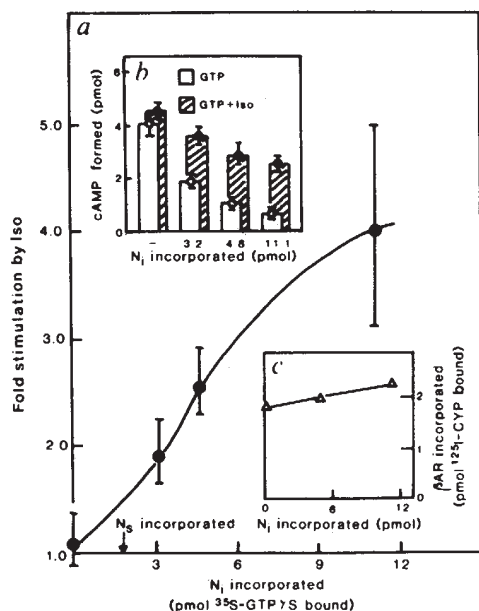


Fig. 2 *a*, Effect of varying N_i on the stimulation (fold) by isoprenaline of the adenylate cyclase activity in phospholipid vesicles. Resolved bovine caudate adenylate cyclase (C) preparations (150 μ l), human erythrocyte N_i (1.16 μ g $\approx$ 12 pmol), human erythrocyte N_i (0.63 μ g, 1.27 μ g, 3.82 μ g $\approx$ 6.6 pmol, 13.4 pmol, 40.2 pmol, respectively) and 12 pmol guinea pig lung β AR were added to reconstitution incubations and protein-lipid vesicles were then formed and isolated as described in Fig. 1 legend. The isolated vesicles were resuspended in 0.38 ml of 75 mM Tris-HCl pH 7.8, 1 mM DTT and assayed (20 μ l) for adenylate cyclase activity (30 min at 30 $^{\circ}$ C) as described in Fig. 1 legend. Isoprenaline (Iso) = 20 μ M, GTP = 0.1 mM. The amount of N_i incorporated into the lipid vesicles was determined by assaying 35 S-GTP γ S binding as described previously¹¹. *b*, Effect of varying N_i on the total adenylate cyclase activity in phospholipid vesicles. The experimental conditions were as described for *a*. *c*, Effect of varying N_i on the incorporation of the β -adrenergic receptor into phospholipid vesicles. Experimental conditions as for *a*. The amount of β AR incorporated into the lipid vesicles was determined by 125 I-iodocyanopindolol (125 I-CYP) binding as described previously¹⁸.

involved in mediating the inhibition of GTP- (or isoprenaline plus GTP-)stimulated adenylate cyclase (manuscript in preparation). This would then suggest that a hormone/receptor-mediated release of the system from inhibition by N_i , as documented here, would be due to the complex between hormone/receptor and activated α_s /GTP becoming insensitive to the inhibitory action of $\beta\gamma$.

We thank Lynn Tilley for her diligence and patience during the preparation of this manuscript, and Drs Allen Spiegel and Robert Somers for the preparation of rhodopsin.

Received 6 June; accepted 17 September 1985.

- Gilman, A. G. *Cell* **36**, 577-579 (1984).
- Houslay, M. D. *Trends biochem. Sci.* **9**, 39-41 (1984).
- Stryer, L., Hurley, J. B. & Fung, B. K. *Curr. Topics Membranes Transp.* **15**, 93-108 (1981).
- Sternweis, P. C. & Robishaw, J. D. *J. biol. Chem.* **259**, 13806-13813 (1984).
- Beer, E. J., Lok, J. M. & Wolf, L. G. *J. biol. Chem.* **259**, 14222-14229 (1984).
- Cerione, R. A. *et al. J. biol. Chem.* **259**, 9979-9982 (1984).
- Brandt, D. R., Asano, T., Pedersen, S. E. & Ross, E. M. *Biochemistry* **22**, 4357-4362 (1983).
- Cerione, R. A. *et al. Biochemistry* **23**, 4519-4525 (1984).
- Asano, T., Pedersen, S. E., Scott, C. W. & Ross, E. M. *Biochemistry* **23**, 5460-5467 (1984).
- Asano, T. & Ross, E. M. *Biochemistry* **23**, 5467-5471 (1984).
- Cerione, R. A. *et al. J. biol. Chem.* **260**, 1493-1500 (1985).
- Codina, J. *et al. J. biol. Chem.* **259**, 5871-5886 (1984).
- Northup, J. K., Smigel, M. D., Sternweis, P. C. & Gilman, A. G. *J. biol. Chem.* **258**, 11369-11376 (1983).
- Katada, T., Bokoch, G. M., Smigel, M. D., Ui, M. & Gilman, A. G. *J. biol. Chem.* **259**, 3856-3859 (1984).
- Hildebrandt, J. D., Hanoune, J. & Birnbaumer, L. *J. biol. Chem.* **257**, 14723-14725 (1982).
- Benovic, J. L., Shorr, R. G. L., Caron, M. G. & Lefkowitz, R. J. *Biochemistry* **23**, 4510-4518 (1984).
- Strittmatter, S. & Neer, E. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6344-6348 (1980).
- Cerione, R. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4899-4903 (1983).
- Cerione, R. A. *et al. Biochemistry* **24**, 4499-4503 (1985).

Regulation of the association of membrane skeletal protein 4.1 with glycophorin by a polyphosphoinositide

Richard A. Anderson & Vincent T. Marchesi

Department of Pathology, Yale University School of Medicine, 310 Cedar Street, PO Box 3333 New Haven, Connecticut 06510, USA

Many of the physical properties of the erythrocyte membrane appear to depend on the membrane skeleton, which is attached to the membrane through associations with transmembrane proteins¹⁻⁵. A membrane skeletal protein, protein 4.1, is pivotal in the assembly of the membrane skeleton because of its ability to promote associations between spectrin and actin⁵⁻⁹. Protein 4.1 also binds to the membrane through at least two sites: a high-affinity site on the glycophorins^{2,10} and a site of lower affinity associated with band 3 (ref. 11). The glycophorin-protein 4.1 association has been proposed to be involved in maintenance of cell shape^{2,12,13}. Here we show that the association between glycophorin and protein 4.1 is regulated by a polyphosphoinositide cofactor. This observation suggests a mechanism which may explain the recently reported dependence of red cell shape on the level of polyphosphoinositides in the membrane¹⁴⁻¹⁶.

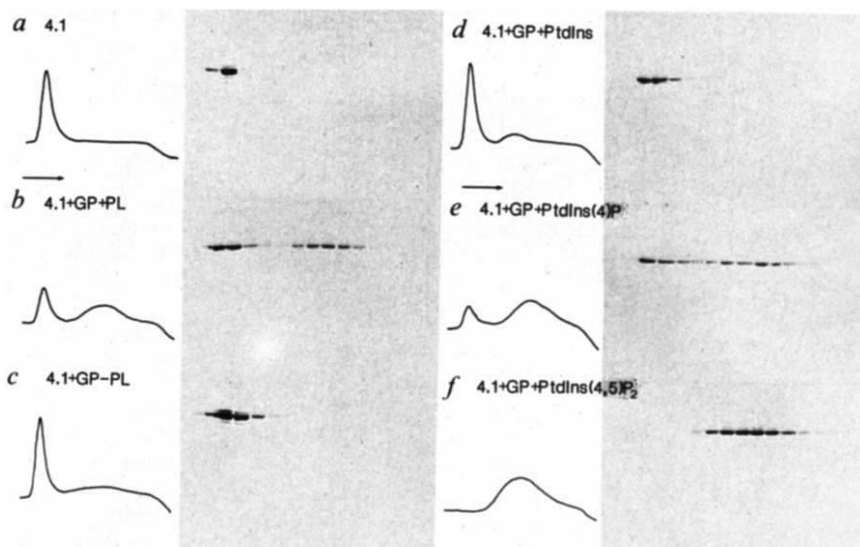
Purified glycophorin, unlike many transmembrane proteins, is soluble in aqueous buffers in the absence of detergents, forming a micellar structure consisting of 14-18 glycophorin monomers with a sedimentation coefficient in the range 7-8S¹⁷. The micellar form of glycophorin varies in its residual intrinsic phospholipid content depending on isolation conditions¹⁸⁻²², but retains the ability to bind protein 4.1. The interaction between micellar glycophorin and protein 4.1 is highly specific and can be used as a method for affinity purification of protein 4.1 (our unpublished results).

Because of the large size of the glycophorin micelles, the complexes of protein 4.1 and micellar glycophorin could be separated easily from free protein 4.1 by density gradient centrifugation. Micellar glycophorin which retained intrinsic bound phospholipid specifically associated with protein 4.1 (Fig. 1*b*), but this ability was lost on extraction of the bound intrinsic phospholipid (Fig. 1*c*). To demonstrate that extraction of bound phospholipid did not irreversibly denature glycophorin, the extracted phospholipids were recombined with glycophorin; glycophorin reconstituted in this way regained its protein 4.1 binding activity, whereas glycophorin treated identically but without added intrinsic phospholipid, did not bind protein 4.1 (not shown).

To determine whether the association between glycophorin and protein 4.1 required a specific phospholipid, glycophorin free of phospholipid was reconstituted with various phospholipids and its protein 4.1 binding capacity studied. Although all the phospholipids were readily reconstituted into glycophorin micelles, only the polyphosphoinositides restored the ability of glycophorin to bind protein 4.1 (Table 1 and Fig. 1*d-f*). Glycophorin forms a stable mixed micelle with phospholipids, probably by hydrophobic interactions between the acyl chains of the phospholipid and the transmembrane segment of glycophorin. The stability of this complex is demonstrated in Fig. 2*a*, in which glycophorin micelles containing 32 P-labelled polyphosphoinositides were analysed by density gradient sedimentation. The polyphosphoinositides completely co-migrate with glycophorin, indicating that the complex is quite stable. Furthermore, on addition of protein 4.1 the sedimentation coefficient of the glycophorin-polyphosphoinositide micelle increases demonstrating formation of a ternary complex. The polyphosphoinositides show a differential reconstitution of glycophorin's protein 4.1 binding activity (Fig. 1*e, f*). Glycophorin micelles containing phosphatidylinositol 4-phosphate

Fig. 1 Sucrose density gradient analysis of the association of protein 4.1 with glycoporphin-phospholipid micelles showing absorbance at 280 nm and corresponding fractions on SDS-polyacrylamide gel electrophoresis (SDS-PAGE). *a*, Protein 4.1 alone; *b*, protein 4.1 plus glycoporphin containing intrinsic phospholipid; *c*, protein 4.1 plus glycoporphin stripped of phospholipid; *d*, protein 4.1 plus glycoporphin reconstituted with phosphatidylinositol (PtdIns); *e*, protein 4.1 plus glycoporphin reconstituted with PtdIns(4)P; *f*, protein 4.1 plus glycoporphin reconstituted with PtdIns(4,5)P₂. The glycoporphin in *c* was treated as in *d-f*, but without added phospholipid.

Methods. Glycoporphin was prepared by the lithium diiodosacrylate/phenol method¹⁸, stripped of phospholipid by extraction with chloroform/methanol/12M HCl (200:199:1)²², and desialated². Desialoglycoporphin was reconstituted with phospholipids by addition of aliquots of glycoporphin (5 mg ml⁻¹) in 60 mM octyl- β -D-glucoside to a dry phospholipid film. The detergent was removed by dialysis, leaving a solution of glycoporphin-phospholipid mixed micelles^{2,27}. The phospholipid content of glycoporphin micelles was quantitated by phosphate analysis^{49,50}. Protein was measured by the method of Lowry using bovine serum albumin as a standard^{2,51}. Purified protein 4.1 (refs 5, 24) and or glycoporphin at a 1:2 molar stoichiometry was applied to a 5–20% sucrose density gradient. The gradients were centrifuged at 40,000 r.p.m. for 16 h in a Beckman SW-40 rotor. The buffer comprised (in mM); 130 KCl, 20 NaCl, 10 Tris, 1 MgCl₂, 0.5 β -mercaptoethanol, 0.1 diisopropylfluorophosphate, pH 7.6 (isotonic KCl buffer).



(PtdIns(4)P) bound protein 4.1 with lower apparent affinity than micelles containing phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂). The association between protein 4.1 and glycoporphin-PtdIns(4,5)P₂ was abolished by treatment of protein 4.1, with *N*-ethylmaleimide, demonstrating that the binding is specific (not shown).

To determine whether the glycoporphin-polyphosphoinositide complex could be a binding site for protein 4.1 on the red cell membrane, we measured the ability of different glycoporphin-phospholipid combinations to compete with membranes for

contrast to the results obtained with glycoporphin micelles reconstituted with other phospholipids (Table 1). Glycoporphin-PtdIns(4,5)P₂ micelles displaced ³²P-protein 4.1 from IOVs more effectively than micelles containing PtdIns(4)P. A plot of the data using the method of Dixon²⁶ indicated that the inhibition of protein 4.1 binding to IOVs is competitive (not shown). Glycoporphin-polyphosphoinositide micelles did not block protein 4.1 binding by direct interactions with the membrane, as IOVs did not bind glycoporphin-polyphosphoinositide micelles and there was no measureable exchange of ³²P-polyphosphoinositide between micellar glycoporphin and the stripped IOVs.

The glycoporphin-polyphosphoinositide micelle contains a specific, high-affinity binding site for protein 4.1 which competes with membranes for protein 4.1 binding. To show that glycoporphin in a membrane environment also requires the polyphosphoinositides as cofactors for protein 4.1 binding, glycoporphin was reconstituted into phosphatidylcholine liposomes together with other phospholipids^{2,27}. We also prepared phosphatidylcholine liposomes containing polyphosphoinositides but no glycoporphin, to determine whether protein 4.1 associates directly with the polyphosphoinositides. ³²P-labelled protein 4.1 bound saturably and with high affinity ($K_a > 10^7$ M⁻¹) to phosphatidylcholine liposomes containing glycoporphin and PtdIns(4,5)P₂. In contrast, liposomes containing glycoporphin and other phospholipids showed only nonspecific protein 4.1 binding. Phosphatidylcholine liposomes containing only PtdIns(4,5)P₂ bound protein 4.1 with low affinity ($K_a < 10^5$ M⁻¹).

The association of protein 4.1 with liposomes containing glycoporphin and PtdIns(4,5)P₂ indicates that the phospholipid requirement of glycoporphin in the membrane is highly specific for PtdIns(4,5)P₂, which suggests that this complex is a protein 4.1 binding site on membranes. However, if this is the case liposomes containing glycoporphin-PtdIns(4,5)P₂ should compete with IOVs for protein 4.1 binding. To study such competition, liposomes were combined with IOVs and competition for ³²P-protein 4.1 binding was measured after separation of IOVs and liposomes by sedimentation through a sucrose cushion (see Fig. 3 legend). In these conditions the liposomes remain in the supernatant whereas the IOVs sediment and are found in the pellet. Analysis of the supernatant and the pellet showed that liposomes containing glycoporphin were uniformly separated from IOVs (Fig. 3a) and that protein 4.1 binding to IOVs was

Table 1 Effect of phospholipid reconstitution of micellar glycoporphin on inhibition of protein 4.1 binding to stripped IOVs

Phospholipid reconstituted	Concentration of glycoporphin (μg ml ⁻¹) at 50% inhibition of 4.1 binding	Moles of phospholipid/glycoporphin*
PtdIns(4,5)P ₂	51	1.11
PtdIns(4)P	115	1.05
Phosphatidylinositol	>400	0.98
Phosphatidic acid	230	0.95
Phosphatidylserine	360	1.05
Phosphatidylethanolamine	>400	0.90
Phosphatidylcholine	>400	0.95
No lipid	>400	0.02

* Mean of three determinations of phospholipid, by phosphate assay^{49,50}, and protein, by Lowry's assay^{2,51}.

protein 4.1 binding. Inside-out erythrocyte membrane vesicles (IOVs) depleted of protein 4.1 were prepared^{23–25} and reconstituted with trace amounts of ³²P-labelled protein 4.1. The ³²P-protein 4.1 appeared to retain complete membrane-binding activity, as over 95% of the labelled protein bound to IOVs or glycoporphin micelles and competed with unlabelled protein 4.1 for binding.

Micellar glycoporphin containing PtdIns(4,5)P₂ or PtdIns(4)P inhibited protein 4.1 binding to stripped IOVs (Fig. 2b), in

Fig. 2 *a*, Sedimentation profiles of 32 P-protein 4.1 ($\circ$), glycoprotein reconstituted with 32 P-PtdIns(4,5) P_2 (Δ), and complexes of protein 4.1 and glycoprotein that had been reconstituted with 32 P-PtdIns(4,5) P_2 ($\blacktriangle$) and 32 P-PtdIns(4)P ($\bullet$). *b*, Inhibition of protein 4.1 binding to stripped IOVs by different glycoprotein-phospholipid mixtures: $\bullet$, glycoprotein stripped of phospholipid; Δ , glycoprotein-PtdIns; $\circ$, glycoprotein-PtdIns(4)P; $\blacktriangle$, glycoprotein-PtdIns(4,5) P_2 ; $\blacksquare$, glycoprotein with intrinsic phospholipid. **Methods.** *a*, Protein 4.1 and polyphosphoinositides were labelled endogenously in IOVs with [γ - 32 P]ATP 52 . The polyphosphoinositides were extracted from membranes with chloroform/methanol/12 M HCl(100:200:1), and the 32 P-labelled PtdIns(4)P and PtdIns(4,5) P_2 were purified on a neomycin B-glycophase G CPG/200 column 53 . The purity of the polyphosphoinositides was analysed by TLC 53 . *b*, Protein 4.1 binding to stripped IOVs and inhibition of binding were determined in isotonic KCl buffer. 32 P-labelled protein 4.1 (3.5 μ g) was combined with stripped IOV (60 μ g of protein) then glycoprotein was added and the suspension was incubated for 1 h at 4 $^{\circ}$ C, then sedimented at 30,000g (30 min) through a 10% sucrose cushion to separate free 4.1 from bound 4.1. The concentration of glycoprotein in the IOVs was estimated from the sialic acid content of the IOVs 2 .

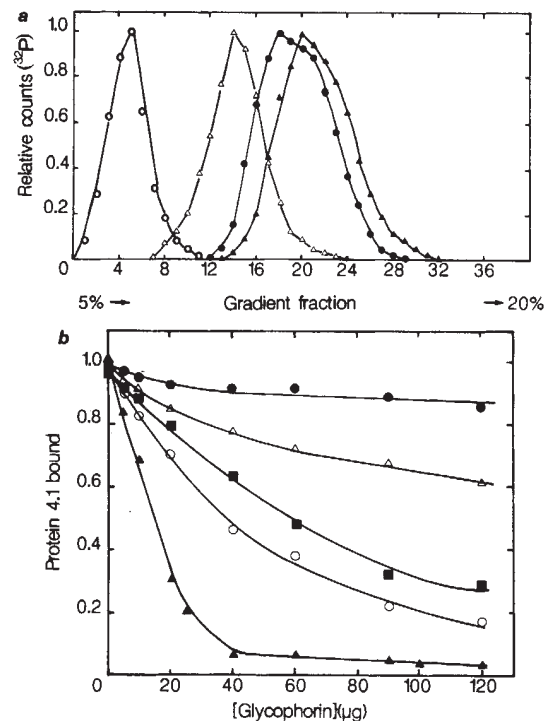
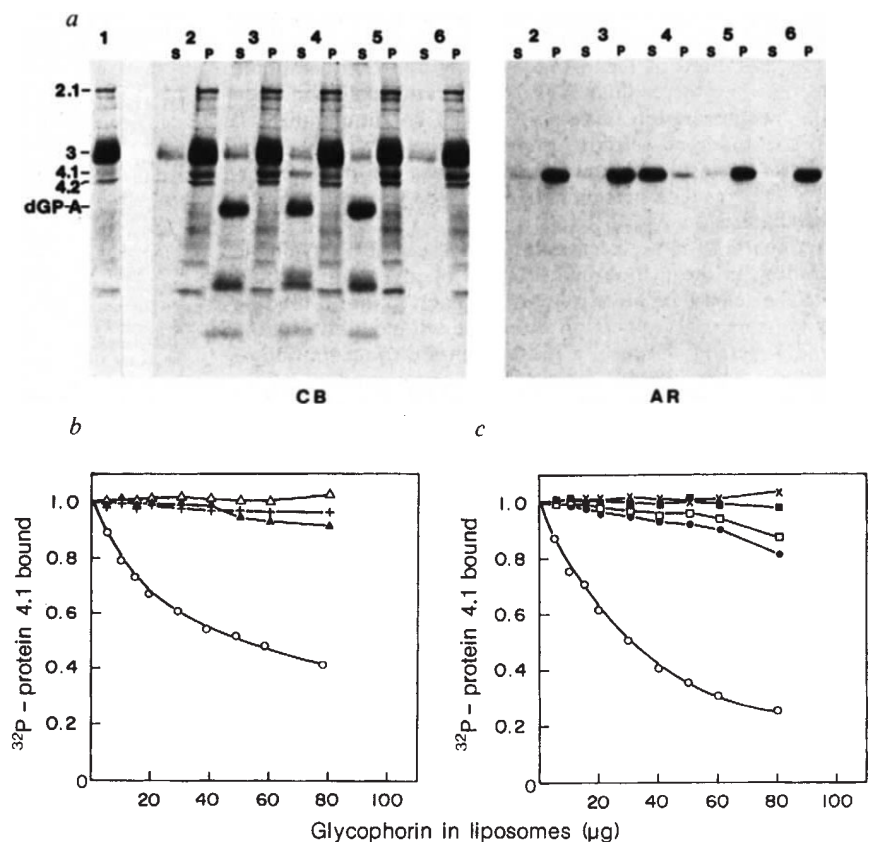


Fig. 3 Inhibition of 32 P-protein 4.1 binding to stripped IOVs by phosphatidylcholine liposomes containing glycoprotein, additional phospholipids, or glycoprotein and additional phospholipids. *a*, 7–15% SDS-PAGE (CB, Coomassie blue-stained; Ar, corresponding autoradiogram) of supernatants and pellets from a qualitative experiment, showing inhibition of 32 P-protein 4.1 binding to stripped IOVs. Lane 1, stripped IOVs; lanes 2, supernatant (S) and pellet (P) of 32 P-protein 4.1 bound to stripped IOVs; lanes 3, as lanes 2 except that liposomes containing desialoglycoprotein (dGP) were added to IOVs (the glycoprotein was pre-stripped of all phospholipid before reconstitution into liposomes); lanes 4, as lanes 2 except that liposomes containing dGP and PtdIns(4,5) P_2 were added to IOVs; lanes 5, as lanes 2 except that liposomes containing dGP and PtdIns(4)P were added to IOVs; lanes 6, as lanes 2 except that liposomes containing PtdIns(4,5) P_2 alone were added to IOVs. *b*, Quantitative inhibition of 32 P-protein 4.1 binding to stripped IOVs by phosphatidylcholine liposomes containing: dGP alone (+), dGP and PtdIns(4)P ($\blacktriangle$), dGP and PtdIns(4,5) P_2 ($\circ$), and PtdIns(4,5) P_2 alone (Δ). *c*, Same as *b*, but phosphatidylcholine liposomes contain: PtdIns(4,5) P_2 and PtdIns(4)P ($\times$); dGP and phosphatidylinositol ($\blacksquare$); sGP and phosphatidylserine ($\square$); dGP and phosphatidic acid ($\bullet$); dGP, PtdIns(4,5) P_2 and PtdIns(4)P ($\circ$). **Methods.** Phosphatidylcholine liposomes were prepared by the detergent dialysis method using octyl- β -D-glucoside 2,27 . Phosphatidylcholine (30 mM), desialoglycoprotein (0.12 mM), other different phospholipids at various concentrations, and octyl- β -D-glucoside (340 mM) were combined in an argon atmosphere, then extensively dialysed (5–6 days) against isotonic KCl buffer flushed with argon 2 . After dialysis the liposomes were layered on a 5–20% sucrose density gradient and centrifuged for 22 h at 200,000g in a Beckman SW-40 rotor. Liposomes containing glycoprotein float at 6–7% sucrose whereas free glycoprotein sediments to the bottom of the gradient. The liposome fraction was collected, dialysed against isotonic KCl, and sedimented at 150,000g for 20 min to concentrate and select for larger liposomes. The molar ratio of phosphatidylcholine (PC) to glycoprotein in the liposome after dialysis was 690 ± 90 mole PC per mol dGP as determined by phosphate 50 and protein 51 analysis. The mol% of added phospholipid to phosphatidylcholine before dialysis was: 0.9 for PtdIns(4,5) P_2 , 1.8 for PtdIns(4)P, 3 for phosphatidic acid, 19 for phosphatidylserine, and 10 for other phospholipids. Stripped IOVs (35 μ g of protein) were recombined with 1.2 μ g of 32 P-protein 4.1 (30,000 c.p.m. μ g $^{-1}$). When quantitating the competitive binding of 32 P-protein 4.1 between IOVs and liposomes, IOVs with bound 32 P-protein 4.1 were combined with liposomes, incubated for 90 min at 23 $^{\circ}$ C, layered over a 10% sucrose cushion, and centrifuged at 38,000g for 30 min. In these conditions IOVs sediment through the sucrose cushion whereas liposomes float at the interface of the cushion. When using liposomes lacking glycoprotein in the binding inhibition assay, the same amount of phospholipid was used as in liposomes containing glycoprotein.



inhibited only by liposomes containing both glycophorin and PtdIns(4,5)P₂ (Fig. 3a-c). Furthermore, quantitation of the competition of ³²P-protein 4.1 binding to IOVs demonstrated that liposomes must contain both glycophorin and PtdIns(4,5)P₂ before they can compete for protein 4.1 binding with IOVs (Fig. 3b, c). Liposomes containing glycophorin as well as other phospholipids did not compete with IOVs for protein 4.1 binding. The fact that liposomes containing up to 19 mol% phosphatidylserine (PS) did not compete with IOVs for protein 4.1 binding demonstrates that the association between protein 4.1 and membranes containing PS²⁸ is of lower affinity than that of the protein 4.1-glycophorin association. Interestingly, liposomes containing glycophorin and both PtdIns(4,5)P₂ and PtdIns(4)P are more effective competitors of protein 4.1 binding to IOVs than liposomes containing glycophorin and PtdIns(4,5)P₂ (Fig. 3c). This result suggests that while PtdIns(4)P by itself does not promote glycophorin-protein 4.1 interactions in the membrane, it may have a synergistic effect on the association of protein 4.1 with glycophorin-PtdIns(4,5)P₂.

Thus, the association between protein 4.1 and the glycophorins appears to be regulated by the polyphosphoinositides. While neither glycophorin nor the polyphosphoinositides bind protein 4.1 in the absence of the other, the association of PtdIns(4,5)P₂ with the cytoplasmic domain of glycophorin forms a high-affinity membrane binding site for protein 4.1. The concentration of the polyphosphoinositides in the membrane is maintained by kinases and phosphatases which are specific for each of the myo-inositol phosphate monoesters^{7,29-31}. The red cell also has a membrane-bound Ca²⁺-activated phospholipase C which is highly specific for the polyphosphoinositides³²⁻³⁴. The concentrations of PtdIns(4,5)P₂ in the red cell membrane decreases when the cellular ATP concentration falls, or when the Ca²⁺ concentration increases; in both conditions the cell membrane undergoes striking physical changes^{12,15,32,33}. Many hypotheses have been proposed to account for this correlation^{12,15,34,35}; the data presented here provide yet another possible mechanism.

A reduction in the concentration of PtdIns(4,5)P₂ available for binding to the cytoplasmic domain of glycophorin could lead to the release of protein 4.1 from its glycophorin binding site, and hence to its relocation within the cell, possibly binding to band 3 (ref. 11). Indeed, a decrease in the concentration of the polyphosphoinositides as a consequence of metabolic depletion or increased cytoplasmic Ca²⁺ concentration resulted in a dramatic decrease in the ability of stripped IOVs to bind protein 4.1 (our unpublished results).

An exciting implication of this finding is an expansion of the role that the polyphosphoinositides may have in cellular regulation. In most cells the polyphosphoinositides are cleaved by phospholipase C in response to stimulation by extracellular ligands that bind to transmembrane receptors. Cleavage of the polyphosphoinositides results in the release of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, both of which may act as second messengers^{29-31,36-38}. However, an alteration of the membrane levels of the polyphosphoinositides may also affect the interaction of membrane proteins with other proteins or enzymes, possibly modifying their intracellular biological activities. In this manner, the polyphosphoinositides may play a more versatile part as membrane-bound phosphorylated cofactors. So far, the only example of this potential regulatory role is the interaction of protein 4.1 with glycophorin. However, analogues of protein 4.1 are found in most cells³⁹⁻⁴⁶. In the brain, the protein 4.1 analogue appears to be synapsin I⁴⁵, a protein which is associated with synaptic vesicles and may play a part in the release of neural transmitters^{47,48}. If synapsin I retains functional as well as structural similarity to protein 4.1, the polyphosphoinositides may also have a key role in the interaction of synapsin I with the synaptic vesicles.

This work was supported in part by USPHS grant R01 GM 21714 to V.T.M. and by a National Research Service Award F32 GM 09184 to R.A.A. We thank Charles E. Mazzucco for technical assistance.

Received 28 March; accepted 18 September 1985.

- Bennett, V. & Stenbuck, P. J. *Nature* **280**, 468-473 (1979); *J. biol. Chem.* **255**, 2540-2548 (1980).
- Anderson, R. A. & Lovrien, R. E. *Nature* **307**, 655-658 (1984).
- Sheetz, M. P. & Singer, S. J. *J. Cell Biol.* **73**, 638-646 (1977).
- Nicolson, G. L. & Painter, R. G. *J. Cell Biol.* **59**, 395-406 (1983).
- Tyler, J. M., Reinhardt, B. N. & Branton, D. *J. biol. Chem.* **255**, 7034-7039 (1980).
- Ungewickell, E., Bennet, P. M., Calvert, R., Ohanian, V. & Gratzner, W. B. *Nature* **280**, 811-814 (1979).
- Fowler, V. & Taylor, D. L. *J. Cell Biol.* **85**, 361-376 (1980).
- Cohen, C. M. & Foley, S. F. *Biochim. biophys. Acta* **688**, 691-701 (1982).
- Ohanian, U. et al. *Biochemistry* **23**, 4416-4420 (1984).
- Mueller, T. J. & Morrison, M. *Erythrocyte Membranes* Vol. 2, 95-112 (Liss, New York, 1981).
- Pasternack, G. R., Anderson, R. A., Leto, T. & Marchesi, V. T. *J. biol. Chem.* **260**, 3676-3683 (1985).
- Anderson, R. A. & Lovrien, R. E. *Nature* **292**, 158-161 (1981).
- Chasis, J. A., Mohandas, N., Mentzer, W., Walker, P. & Shohet, S. B. *Blood* **25**, (1983).
- Michell, R. H. *Biochim. biophys. Acta* **415**, 81-147 (1975).
- Ferrell, J. E. & Huestis, W. H. *J. Cell Biol.* **98**, 1992-1998 (1984).
- Allan, D. & Thomas, P. *Biochem. J.* **198**, 433-440 (1981).
- Furthmayr, H. & Marchesi, V. T. *Meth. Enzym.* **96**, 268-280 (1983).
- Marchesi, V. T. & Andrews, E. P. *Science* **174**, 1247-1249 (1971).
- Van Zoelen, E. J. J., Zwaal, R. F. A., Reuvers, F. A. M., Demel, R. A. & Van Deenen, L. L. M. *Biochim. biophys. Acta* **464**, 482-492 (1977).
- Buckley, J. T. *Can. J. Biochem.* **56**, 349-351 (1978).
- Shukla, H. D., Coleman, R., Finean, J. B. & Michell, R. H. *Biochem. J.* **179**, 441-444 (1979).
- Armitage, I. M., Shapiro, D. L., Furthmayr, H. & Marchesi, V. T. *Biochemistry* **16**, 1317-1320 (1977).
- Speicher, D. W., Morrow, J. S., Knowles, W. J. & Marchesi, V. T. *J. biol. Chem.* **257**, 9093-9101 (1982).
- Tyler, J. M., Hargreaves, W. & Branton, D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5192-5196 (1979).
- Hargreaves, W. R., Giedd, K. N., Verkleij, A. & Branton, D. *J. biol. Chem.* **255**, 11965-11972 (1980).
- Dixon, M. *Biochem. J.* **55**, 170-171 (1953).
- Mimms, L. T., Zampighi, G., Nozaki, Y., Tanford, C. & Reynolds, J. A. *Biochemistry* **20**, 833-840 (1981).
- Sato, S. B. & Onnishi, S. *Eur. J. Biochem.* **130**, 19-26 (1983).
- Berridge, M. J. & Irvine, R. F. *Nature* **312**, 315-321 (1984).
- Downes, C. P. & Michell, R. H. *Cell Calcium* **3**, 467-502 (1982).
- Irvine, R. F. *Cell Calcium* **3**, 295-309 (1982).
- Allan, D. & Michell, R. H. *Biochim. biophys. Acta* **508**, 277-286 (1978).
- Downes, C. P. & Mitchell, R. H. *Biochem. J.* **198**, 133-140 (1981).
- Allan, D., Thomas, P. & Michell, R. H. *Nature* **276**, 289-290 (1978).
- Sheetz, M. P. & Singer, S. J. *Proc. natl. Acad. Sci. U.S.A.* **71**, 4457-4461 (1974).
- Berridge, M. J. *Biochem. J.* **220**, 345-360 (1984).
- Majerus, P. W., Neufeld, E. J. & Wilson, D. B. *Cell* **37**, 701-703 (1984).
- Nishizuka, Y. *Nature* **308**, 693-698 (1984).
- Cohen, C. M., Foley, S. F. & Korsgren, C. *Nature* **299**, 648-650 (1982).
- Spiegel, J. E., Beardsley, D. S., Southwick, F. S. & Lux, S. E. *J. Cell Biol.* **99**, 886-893 (1984).
- Goodman, S. R., Casoria, L. A., Coleman, D. B. & Zagon, I. G. *Science* **224**, 1433-1438 (1984).
- Granger, B. L. & Lazarides, E. *Cell* **37**, 595-607 (1984).
- Aster, J. C., Welsh, M. J., Brewer, G. J. & Maisel, H. *Biochem. biophys. Res. Commun.* **119**, 226-234 (1984).
- Granger, B. L. & Lazarides, E. *Nature* **313**, 238-241 (1985).
- Baines, A. J. & Bennett, V. *Nature* **315**, 410-413 (1985).
- Leto, T. L., Pratt, B. M. & Madri, J. *Fedn. Proc.* **44**, 1600 (1985).
- Navone, F., Greengard, P. & DeCamilli, P. *Science* **226**, 1209-1211 (1985).
- DeCamilli, P., Cameron, R. & Greengard, P. *J. Cell Biol.* **96**, 1337-1354 (1983).
- Bartlett, G. R. *J. biol. Chem.* **234**, 466-468 (1969).
- Duck-chong, C. G. *Lipids* **14**, 492-497 (1979).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).
- Leto, T. L. & Marchesi, V. T. *J. biol. Chem.* **259**, 4603-4608 (1984).
- Sacht, J. *J. Lipid Res.* **19**, 1063-1067 (1978).

Erratum

Flow law for ice in polar sheets

W. S. B. Paterson; Reply by C. S. M. Doake & E. W. Wolff
Nature **318**, 82-83 (1985)

AN error in *Nature*'s editorial office led to the use of an uncorrected proof for W. S. B. Paterson's contribution to this Matters Arising item. In line 5, the word 'constant' should read 'parameter'. The first sentence in paragraph 3 should read '(2) At Camp Century, the strain rate measured for $\tau_{xz} = 10$ kPa, where x and z define a regular coordinate system, is less than the observational error⁴. In the address, the postcode should read 'Canada VOP 1H0'.